This is to certify that the
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SOLID STATE BIOREACTOR METHOD
FOR PRODUCTION OF
COMPOST WATER EXTRACT

presented by

Michael-Salomon Jost

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of the requirements for the

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SOLID STATE BIOREACTOR METHOD FOR PRODUCTION OF COMPOST WATER EXTRACT

By

Michael-Salomon Jost

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ABSTRACT

SOLID STATE BIOREACTOR METHOD FOR PRODUCTION OF COMPOST WATER EXTRACT

By

Michael-Salomon Jost

Aerated compost tea (ACT) is applied to either the root zone or leaf surface of plants to support or improve the biological diversity, the host resistance to pathogens, and or the nutrient status. Compost, water, and amendment(s) are maintained under conditions conducive for biological process for 24 to 72 hr before course filtering and application. Rather than increase bacteria, fungi and other organisms in a liquid phase, the compost was amended and cultured to increase microorganisms in the solid phase prior to a 20 minute water extraction. A 0.5 L bioreactor was developed to provide reliable control of temperature, gas flow, oxygen levels, and moisture content of the media amended with an alfalfa based substrate (alfalfa meal, poultry protein, potassium sulfate, molasses) as an available energy source for the resident micro flora. The biomass of fungi and bacteria, enumeration of bacteria and nematodes, genetic signature of the bacteria, pH and electrical conductivity, moisture content, of carbon dioxide and oxygen, gas concentration (%), gradient (%), and respiration (g), of carbon dioxide the hour of half maximum and the total potential maximum respired, and volatile compounds released over the incubation period were quantified. Results from MSU and independent labs provided consistent data between replicates within a study and as well between studies with the same parameters. Manipulation of oxygen (3% vs. 20%) and temperature (25 °C vs. 40 °C) influenced the rate of substrate decomposition and the characteristics of the final water extract.

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than thirty years ago introduced the dynamics of compost and how to go about understanding the beast.

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Introduction

The basic hypothesis guiding this thesis research are: A) the mode of action of protection or suppression of plant pathogenic diseases or herbivorous insects by compost "tea" or water extract is not known, although several possibilities have been proposed and are being studied; B) in order to effectively elucidate a mode or modes of action, a definable and reproducible method of preparing compost and compost "tea" needs to be determined; C) it would be advantageous if a variable range of beneficial organisms could be obtained in "tea" from a single source of compost depending on the extraction protocol; and D) that there likely is a identified organism that would be more effective in managing a specific disease or insect, for example based on the production method of compost tea or extract under anaerobic or aerobic conditions, or the microbial populations of ether bacteria or fungi. Assumption D is not a focus of this research because in order to test the assumption, a reproducible protocol must first be established. Therefore, the focus of this literature review is to determine the existing variables in the composting and compost "tea" making process that can be characterized and regulated. The order of the review is to first address compost as a product and composting as a process, then to address compost tea as both a product and a process, and finally to look at manageable variables in both processes.

Compost

Understanding compost is facilitated by considering both the finished product called compost and the process called composting (Rynk, 1992). Also important is to consider what terms and definitions are used to describe the characteristics of compost and existing regulations to insure standardization in the market.

Compost as a Product

Compiling the introductory comments of several authors (Cooperband, 2002; Nilsson, 1994; Resource Recycling Systems, 2002; Rynk and Richard, 2001; Tremorshuizen, 2004) there is an overarching consensus to what the process produces (compost) and the benefits of compost application in plant production systems despite it being a highly variable product. Composting takes place on various scales: industrial, farm, or in the back yard. It is a treatment method that transforms raw organic waste into an excellent soil amendment. The degradation of the materials is carried out by the microbial activity within the decomposing organic waste. In the process of consuming the raw materials by microorganisms, a portion is released as byproducts back into the environment, while that which remains is a stabilized product that is made up of complex compounds and physical properties. When managed correctly there are no problems associated to the environment or within the product. If not, a multitude of problems occur resulting in several negative attributes effecting the air with disagreeable odors, water run off laden with salts and compounds, and soil saturated with leachate.

A few of the primary reasons for composting is the sanitation of materials by a self heating thermophilic phase and the end result as a stable form of organic matter that contributes to long term fertility. The organic matter content in the soil is important for both physical and biological processes. This product when applied to the soil and becomes biologically integrated resulting decreased bulk density, increased aggregate stability, porosity, root penetrability, water retention, nutrient holding capacity, and contributes to buffer capacity. Biologically it effects the indirect nutritional support of both microbes, animals, and plants.

The use of compost as part of a fertility program for cultivated soils provides positive benefits (Stoffella and Kahn, 2001) in the commercial vegetable cropping systems, ornamental and nursery systems, landscaping, fruit production, sod production, and turf management. A few of the direct benefits to the soil, and subsequently the cultivated plants, are a reduced bulk density, an increase in organic mater, water retention, microbial diversity, and long term fertility. In organic farming the use of compost to improve plant health is an integral part of both soil fertility and disease and insect management plans. Compost can also be used as a component in potting or container plant media (Fitzpatrick, 2001).

Of particular interest in this study is the use of compost in plant disease management. Litterrick et al. (2004) in a review paper identified pathogen suppression as a result of the application of compost directly to the soil. Suppression of 43 different diseases on 60 crops is reported in 78 scientific peer reviewed publications (1979-2003). Litterrick et al. (2004) describe four mechanisms of disease suppression by soil applied compost are induced protection, hyperparasitism, antibiosis, and competition for nutrients. For a few specific pathogens, protocols have been developed (Hoitink, 1996) for predicating the suppression of compost made of tree bark and light sphagnum peat, based on the amount of available carbon. The protocol does not provide insights to the duration of the suppressive qualities are to be expected. As the soil biology, more specifically bacteria, are dependent either indirectly or directly on the organic mater in the soil, the feed stock is classified (Henis, 1987a) by the range that is available for the degradation by microbes. Three groupings are made, the first, as the fresh plant material that are readily available, followed by substances with a half life of five to twenty five

years, and lastly the very stable organic material that is resistant in the range of two hundred fifty to five thousand years.

Composting as a Process

The process of composting has a long history closely associated with cultural practices in agriculture and soil fertility. Currently the use of the composting process also encompasses the management of organic wastes, both in industrialized production systems and municipal waste. A great wealth of literature in book form (De Bertoldi et al., 1996; De Bertoldi et al., 1987; Martin, 1992) is available for a wide range of studies and application for the process of composting.

Composting involves the collection and placement of organic materials amassed to allow decomposition. Controllable factors for composting are: organic amendment, carbon to nitrogen ration, particle size, percent recycled compost, mixing equipment, reactor vessel size, stirring frequency, chemical pH moderating agent, initial moisture content, temperature, moisture control aeration, ambient temperature, retention time, depth, percent recycled air, type of process, curing time, inoculation, and bulking agents (Hansen, 1989). After the initial setup of the pile there are several ways that the material can be manipulated to influence the composting process. Manipulation includes, but certainly not limited to: mixing, aeration, moisture management, and temperature regulation. Of the several goals to be met in the process of composting, one is that the mixture of materials is to provide for microbial development that leads to an increase of temperature within the pile. This process of self heating can peak at 45-70 °C within three days and provides for the reduction of weed seeds and resident plant, human or animal pathogens within the materials (Epstein, 2001). Influencing this increase in

temperature is the ratio of carbon to nitrogen, porosity/aeration, moisture content, and the size of the pile. After temperature has peaked it is maintained by continued self heating for a period of time which then is followed by incremental temperature decent when the material no longer has readily available energy sources for the microbes. At the end of this phase the material has changed in volume, texture, appearance, odor, resident microbial population, and stability. Depending on the targeted use, the product is usually stockpiled for additional curing.

Compost Quality

Producers of compost concerned with the quality of the product need to establish at what point in the process the product is considered fit for use and meets the requirements set by the end user. There is no one size fits all determinant. For example, a site processing municipal wastes may be concerned with human pathogen levels within the product and space limitations to allow curing of piles. An industrial scale facility based on the composting of agricultural byproducts for garden application, may be concerned with the density and reheating within the shipping bags through to the eventual retail sale. Concerns of the use of compost in potting media for container plant production include the porosity, pathogen suppression, and reduction of volume over time. On farm production, using in-house materials that are to be used on site, may be concerned with the crop's requirements so as to enhance either vegetative or reproductive growth. The physical properties, including particle size and moisture content, must allow for uniform application at relatively low rates per unit area, often with lime/fertilizer spreading equipment.

Five overarching quality indicators (Ingham, 2003; Stoffella and Kahn, 2001) include that the process of composting and the product compost with the intended application to promote plant health are: 1) underwent a thermophilic phase for significant reduction of resident weed seed and pathogens, 2) has not undergone prolonged anaerobic conditions, 3) has reached a point of stability or maturity that self heating is not evident and turning or mixing to meet oxygen demand is no longer necessary (and that this condition is not simply due to a lack of moisture), 4) has a low enough moisture content to allow easy handling and that restricted gas exchange does not lead to the development of anaerobic microbes and compounds during curing, and 5) contains a large and diverse population of desirable microorganisms.

Sullivan and Miller (2001) summarized a large number of methods and protocols developed by others to assist the producer and end user to evaluate the compost. Simple tests include the particle size distribution, soluble salts, and stability as indicators of the potential product end use. Maturity refers to the possible use of the compost and stability is the rate of degradation of the organic mater over time. Indicators for the maturity are based on sensory observation, chemical, and/or phytotoxicity test. Stability is a relative measure of remaining available carbon established by respirometry and/or the Dewar self-heating test (Brinton Jr. and Brinton, 1995).

In order to mitigate the misinterpretations based on only one or two tests a recently formulated index (Buchanan, 2002) makes use of the testing methods that are used for compost. By using a few of the selected tests in a specific series of steps, compost is evaluated as immature, mature, or very mature. It also takes into consideration the final use, location and the rate of application of such materials.

Independent of use, the physical properties are described by evaluating the moisture content, bulk density, water-holding capacity, particle size, or man-made inerts such as plastics

Chemical properties are evaluated by total organic carbon, volatile solids, humic substances, cation exchange capacity, total nitrogen, inorganic nitrogen, acidity/alkalinity, electrical conductivity, phosphorus, potassium, calcium, magnesium, and micronutrients. Biological tests are done by direct measurements and number counts of microbes, microbial biomass, plate colony forming units, suppressive capacity bioassay test (Mandelbaum, 1997), and polymerase chain reaction (Schwieger, 1998; Trescher, 2002).

Compost Regulations

The United States Environmental Protection Agency (EPA) has published (Environmental Agency (EPA) Technology Transfer, 1985) guidelines and requirements defining the process of composting and compost as a product. The primary concern for the regulation is to address the control of pathogens afflicting humans. United States Department of Agriculture has specifically addressed the definitions and use of compost in organic agriculture as outlined by The National Organic Program (NOP) (2000). Under the NOP rule the requirement in the composting protocol is that the initial C:N ratio of the mixture of materials is to be between 25- 40:1 and that if the ensuing decomposition takes place within an in-vessel or static aerated pile, self heating temperatures must be maintained between 45-70 °C for three days. In the case of windrow systems the same target temperature must be maintained for fifteen days and turned at a minimum of five times. Currently the standards advisory board (NOSB) to

the NOP has put forth a proposal to amend the rules with a standing EPA regulation (Environmental Agency (EPA) Technology Transfer, 1985) that requires that the entire pile heats above 54.5 °C for at least 3 days and no specified number of turning is required.

Compost Tea and Extracts

Compost tea as a product

Litterrick, et al. (2004) reviewed cultural practices for sustainable forms of agriculture and horticultural in the temperate zone. The reduction of both pest/disease incidence and severity as result of application of uncomposted materials, compost, manures, compost extract and tea is presented. Great lengths are taken in the clarification of former interchangeable and current usages of the terms related to compost tea. Compost tea is described by Litterrick, et al. as a filtered product that is manufactured by placing compost in water under either aerobic or anaerobic conditions. This difference in the processes leads the product to be referred to as being either aerated (ACT) or non-aerated (NCT) compost tea. Although not always having been defined in the following way, compost extract (CE), is referred to as a filtered product that is of a compost water mixture that has not been aerated or fermented.

Noteworthy within the review (Litterrick, 2004) is that of the published results up until now using compost teas has been done primarily with NCT. The authors are unable to make a general statement from research reports using ACT as there are few results from the papers reviewed (3 of 25) and results varied greatly. They attribute this to experimental design, environmental factors, or crop specificity using ACT. Despite these short comings, examples of proven disease suppression of 20 pathogens on 25

crops are reported in 29 scientific peer reviewed publications (1988-2003). Currently the overall direction of development by a large number of individuals in the USA is the consensus that the process is best done under aerobic conditions so as to avoid bad odors and phytotoxic metabolites.

In the trade journal The IPM Practitioner (Quarles, 2001) production volume is cited as an independent variable that needs to be scaled to meet the needs of the operation in the article: Compost Tea for Organic Farming and Gardening. Also addressed in this article is that although the primary intent of the use of compost tea is to target directly foliar pathogen development, the nutrient component of the applied substance can also be part of a foliar feeding program. The article address the interaction between a biologically derived product and diseases citing several references where the interactions have positive out comes. Results, not limited only to compost tea, obtained by researchers in lab and in field settings with various process of production, treatment (sterilization, and sterilization and re inoculation), and application methods are presented. Based on the results of one study (Zhang, 1998) a partial explanation into the mechanism is presented as cucumber plants treated with a foliar application of compost water extract responded to an induced infection by an increase within the plant of a compound associated with an acquired systemic resistance.

Further detailed information specific to compost tea and extracts and the most current production methods, application, and regulations is available at the web site http://attra.ncat.org/, hosted by Appropriate Technology Transfer for Rural Areas (ATTRA) of the National Sustainable Agriculture Information Service. Therein are listed plant disease control, science papers and links posted on the world wide web,

literature reviews, e-mail discussion lists, National Organic Standards Board (NOSB) tea task force report, Human Pathogens from livestock Manure - proceedings, on-farm research into compost teas, compost tea substrates, additives and supplies, laboratories that specialize in Microbial Analysis of compost teas, and compost tea resources from Soil Foodweb, inc.

Compost Tea Preparation Process

Quarles et al. (2001) provides an overview citing several references to the practices of the manufacturing and application of compost tea. In order to differentiate from "brewed compost tea" and other easily mistaken forms of "tea", "extracts", and "leachates", a quick overview of the production processes follows. "Manure tea" is produced by suspending animal manure in water and then applying it to the soil or growing plants. "Compost extract" (Weilzien, 1991) is the result of either, running water through, or soaking in water, a volume of compost to extract the microbes and compounds from the compost. "Compost leachate" is the water that drains off of a water saturated compost and is primarily soluble materials and nutrients.

Currently receiving attention in the USA is the production and use of "Brewed Compost Tea". In this particular process, compost is suspended in a container within a vessel filled with a nutrient-water solution for microbial growth. Recommended brew time based on the microbial diversity (DNA) and total biomass (direct count) of organisms (no data values given for either) development to be optimum between 12 and 48 hours. Under the following headings (Application, Production site, Vessel, Water, Amendments, and Compost) the materials used (compost, nutrient amendment, and

water), and the tools for production and application of "Brewed Compost Tea" (BTC) will be covered as recommended by Ingham (2003).

Compost. The starting material to be used is mature compost. The ratio of compost to water varies depending on the vessel producer's recommendations. On a volume to volume basis the ratios range from 0.3:10 to 2.5:10 with 1:10 most common.

Amendments. An available energy source is needed for exponential growth of the microbial population during the process. The current approach recommends that at the beginning of a brew cycle an amendment is added. Depending on which type of microbial growth desired (fungal or bacterial) specific types of materials are added. Growers can either use ready made nutrient/biological mixes prepared by commercial labs, or make use of products such as simple sugar, plant extracts, fatty acid, cellulose waxes, and humic acid.

Water. The water to be used has few requirements. In the most general terms it must not contain high levels of salts, nitrate, heavy metals, sulfur, tannic acid, carbonates or disinfectant materials such as chlorine. This also applies to human or animal pathogens. It is recommended that the water be tested at a lab and steps taken to correct the levels of contaminates. The specific values and ranges are not given. An alternate proposed water test is outlined in the manual that will not be addressed in this paper based on the subjectivity and the risks involved. The water is to be warmed +/-1.5 degrees of 20 °C.

Vessel. The brew vessel is where the process occurs. The complexity of the vessel range from a bucket with an aquarium bubble system to a commercially engineered brewer. Size varies from 5 to 1000 gallons. The vessel is first filled with

water followed either with the addition of amendment or compost. Emphasis is placed on maintaining dissolved oxygen levels at or above 5.5 ppm with "clean" atmospheric air through the duration of the brew cycle. Some systems have pumps that continuously or intermittently circulate the liquid throughout the bioreactor others rely on the upward movement of the injected air to provide movement.

Production site. The location where the brewer is set up during the production cycle is to be conducive in buffering the ambient sunlight and temperature so that the brew does not undergo great fluctuations.

Application. Brewed Compost Tea is recommended to be applied either at full strength or diluted with water. When preparing a dilution, surfactants, stickers, or nutritive products can be mixed with the water before the addition of the Brewed Compost Tea. Nutritive products are used with the intent to either provide the newly applied microbes an energy source for further development or as a foliar nutrient application. Brewed Compost Tea is customarily applied on to the soil or the aerial structure of plants as a drench or spray using, for example large volume tanker, hand-whisk, or a conventional spray rig. Recommend within the manual is to take into consideration that the velocity and/or pressure that is applied to the suspension can be detrimental to the microbial population.

Evaluation or Characterization of Compost Tea Quality

Currently in the USA, there are few labs (Soil Foodweb, inc., 1750 SW 3rd St Suite K, Corvallis OR; Microbial Matrix Systems, Inc., 33935 Hwy 99E, Suite B, Tangent, OR 97389) that specialize in the evaluation or characterization (Ingham, 1982; Ingham, 1986; Weaver, 1994) of compost and compost tea. In contrast to other labs,

either at universities or within the microbial industry at large, these labs make an interpretative evaluation (based on a proprietary data base) and make recommendations for use in agriculture. The labs suggest that practitioners send in samples of the product periodically to gain an understanding in the trends in what and to verify that that their target goals of the microbial populations are met. Using direct microscopy the recommended benchmark values (exceeding the mark is not seen as detrimental in all but two cases) of organisms in one milliliter of compost tea are as follows. The microgram biomass target range are 10 to 150 active bacteria, 150 to 3000 total bacteria, 2 to 10 active fungi, and 5 to 20 total fungal. A minimum of 10% of the total bacteria or fungi must be active. The target number of individual microbes are 2000 protozoa (1000 flagellates, 1000 amoebae, 5 to 10 ciliates), and 2 to 10 beneficial nematodes (1 to 5 bacterial feeders, 0 to 5 fugal feeders, 1 to 5 predatory, no root-feeding nematodes). Using molecular methods the target number of species for bacteria is 15000 to 25000 and for fungal 5000 to 8000.

Regulatory Statutes

The National Organic Standards Board established the Compost Tea Task Force to address concerns regarding the application of these forms of extracts and preparations for use in pathogen control on crops intended for human consumption. The task force published report (National Organic Standards Board, 2004) covers and defines a wide range of concerns that arise starting from the of quality of compost to be used and the initial fabrication set up all way through to the number of pre harvest days needed after application. Although not the intent of this research project to explore the facet of non intended consequences concerning human health, helpful are the definitions of the

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products and protocols with a focus of the "...concern about the potential for compost tea to contaminate edible plants with human pathogens as regulated in Section205.203 of the USDA National Organic Program Final Rule". Further denoted within the report the product definition of compost tea and compost extract, is established by the method of production. They are defined as, "...this report considers any mixture of compost and water that is held for longer than one [1] hour before initiating application to be a form of compost tea. Any mixture of compost and water that is held for less than one hour before initiation application is considered a compost extract" (National Organic Standards Board, 2004).

Using regulatory statues from the US Environmental Protection Agency (EPA) definitions and testing methods have been incorporated into the recommendations to address human pathogen concerns. Product defined and produced as compost tea that are made without any additives or a compost extract (any mixture of compost, water, additives, and adjutants) can be applied without restrictions. Compost tea made with additives must have at least two replicated batches tested using EPA standards and tests for human pathogens. Having tested negative, using the identical protocol, it can be applied without restriction. Significant to the research presented here is that the use of these products has reached a frequency that regulatory institutions have taken note.

Factors Influencing Microbial Development in Compost

There are at least seven factors that can be regulated (Hansen, 1989), including temperature, moisture, substrate/feedstock C:N ratio, substrate/feedstock particle size, aeration, pH, and the microbes present or introduced. In addition, all the preceding variables can be influenced by the site of composting and management of the pile.

Temperature

The effect of high temperature on living forms has been studied on a macro scale from environmental conditions world wide. In a summary review (Brock, 1978) the upper temperature limit for various living organisms are stated. For vascular plants it is 45 °C and mosses have an extended range to 50 °C. Protozoa have an upper limit of 56 °C, algae 55-60 °C, fungi 60-62 °C. Prokaryotic microbes such as blue green algae are able to survive up to 70-73 °C followed by chemoolithotrophic and heterotrophic bacteria capable surviving ranges greater than of 90 °C. From studies made on the waters flowing off hot springs, the effects of temperature increases and the non linear relation to effects on organisms serve as an example. Beetles species adapted to hot spring waters sustain reduction in numbers (33, 22, 15, 10, 6) as the temperature (39, 40, 41, 42, 43 °C) increases. A similar trend is found with the number of blue-green algae species (42, 54, 76, 86, 90, 86, 76, 60, 25, 24, 2, 1, 1) and temperature (15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 °C).

During hot composting (> 40 °C) there are changes of internal temperatures associated with particular phases of decomposition. A characterization of the changing (Day, 2001) temperatures have been allocated to three phases. The initial phase after the establishment of the compost pile is that within 24-48 hours the internal temperature increases from ambient to the range of 40-50 °C. The second phase is associated with temperatures of 55-70 °C that are attained within several days of the preceding increase. The duration of these elevated temperatures can be for a few days to several weeks. The final phase characterized is associated with the decline in production of heat over several

weeks from the peak temperature all the way through to once again the internal temperature is that similar to that of soil.

The effect of temperature on the capacity for specific species to sustain a population has prompted the classification of groups within ranges. Within compost the definition and general temperature range are (Day, 2001) given to microorganisms as cryophiles or psychrophiles in the 0-25 °C, mesophiles range of 25-45 °C, and thermophiles having a range greater than 45 °C. Others (Henis, 1987a) make the classification for a wide range of environments in addition to those of compost as cryophiles or psychrophiles in the range of -7 and 25 °C, mesophiles min/max of 10 and 40 °C (optimum in the 20-30 °C), and the thermophiles with These ranges established a minimum temperature of 30 °C and a max of 70 -80 °C (optimum in the 55-60 °C with an additional note: obligatory thermophiles can not grow under 30 °C.)

Temperature dynamics of the compost mass is attributed (Moubasher, 1984) to the exothermic reactions of mesophilic microflora. As the temperature elevates to the vicinity of approximately 40 °C, spores of the thermophilic organisms germinate and elevate the temperature out of the mesophilic range towards 60 °C. During the subsequent phase, the zones (Hoitink, 2001) where the temperature decline from the peak and become lower than 40 °C, mesophilic organisms re-colonize the material from those that are already lower in temperature.

Keeping to over arching generalities of the bacterial and fungal developments (Day, 2001) through the process, 80-90% of the microbial activity is attributed to that of bacteria and in most cases bacteria numbers are a 100 times more prevalent than fungi. At the start of composting, there are bacteria populations with a large number of

Streptococcus, *Virio* and *Bacillus* with at least 2000 strains. The mesophilic stage contained *Bacillus*, *Psuedomonas*, *Arthrobactre*, and *Alicalegenes* as precursors to the thermophilic stage where 87% were identified as *Bacillus subtilis*, *B. streatothermophilic*, and *B. Licheniformis*. Also reported was that as temperature increased the bacterial colony variety decreased.

From compost there are more types of fungi identified than that of bacteria. There are two general forms of fungi made up of yeasts and molds. Common fungi are *Aspergillus*, *Penicillin*, *Fusarium*, *Trichoderma* and *Chaetomoniun* (Day, 2001) . Kane and Mullins (1973) identified 304 fungi (unifungal cultures: Mucor, 120; Aspergillus, 97; Humicola, 78; Dactylomyces, 6; Torula, 2 Chaetomium, 1) under elevated temperatures from a high rate municipal composting system. The selected isolates grown on Emreson YpSs agar, were found to have the greatest growth rates at 40–55 °C and none were able to grow in temperatures greater than 60 °C. Samples grow on the agar collected from the compost where temperatures greater than 70 °C produced sparse growth. Day and Shaw (2001) summaries reports by others as that mesophilic fungi have an upper temperature of 45 °C and that the thermophiles have an upper limit of activity at 60 °C. Others report (Henis, 1987a) mesophiles have an upper limit of 40 °C and that some thermophiles are defined as those with the inability to grow at temperatures below 20 °C and having a maximum rate of growth at either or above 50 °C. Certainty on the upper temperature limit for fungi has been set in the pasteurization of compost for use within a study (Tuomela, 2000) and within the industry of button mushroom production. The material was rendered with no fungal activity after maintaining the temperature at 70 °C for 4 hours.

As temperature increases over 45 °C there is a reduction of fungal species. In the introduction of a study involving thermophilic microbes (Maheshwari, 2000) it is highlighted that there are few species of fungi that have the capacity to thrive in the temperatures within the range of 45 to 55 °C. Of the 50,000 known fungal species there are 30 some species known to function above of 55 °C. In studies using wheat straw compost (Moubasher, 1984) total fungal count reduction occurred on day 1-3 with internal temperatures at 53.4-59.0 °C. For thermopiles the conditions required to support their development are warmth, humidity and an aerobic environment.

As the temperature decreases from thermophilic conditions the monitoring of populations (Klamer and Baath, 1998) of microbes in a compost of straw and pig slurry demonstrated that on day 10-20 after setting up the compost, Gram negative bacteria and fungi increased as temperatures decreased to 40-50 °C after the initial peak. The reappearance of microorganisms is referred to as thermodurism (Henis, 1987a; Henis, 1987b). Some microbes demonstrate the ability to withstand high temperatures and return to normal functions after temperatures have reduced. The increase in temperature is not only a selective process but also meets specific requirements of development for fungi.

Reported observations in regards to germination and growth of hyphae are summarized (Maheshwari, 2000) as once spores have germinated with high temperature, these temperatures are no longer required; hyphae growth is 10-12 °C lower. Also noted by the same group of authors is that thermophilic fungi are most active at high temperatures and yet in a study using a single cultured fungus a range of functionality was established as it exhibited continued growth until the temperature was lowered to 31

°C and yet respiration would remain unchanged. It was only with the reduction to 21 °C respiration stopped after 24 hours.

Under laboratory conditions a specific species of fungi grow equally well on media based on cellulose or glucose (Maheshwari, 2000). On the other hand the use of either cellobiose or cellulose based media mix resulted in either strong growth or the mycelia autolysed and sporulated respectively.

Composition: Carbon and Nitrogen Ratio and Availability

Microbes require nitrogen to synthesize proteins and carbon to supply energy (Horwath, 1996). The carbon, nitrogen, and their ratio influence microbial development and metabolic byproducts of both the microbial populations and the composting process. For successful composting (Day, 2001) the C:N ratio can be as wide as 17-78:1, though it is a ratio of 25-35:1 that inherently facilitates the overall ease of the composting process. Farmers and composting facilities are concerned with the ratio as it influences the overall nutrient balance of the farm and impacts the environment of the local production site. As finished product compost needs to have a C:N ratio of 25-30:1 in order to prevent nitrogen immobilization by microbes away from growing plants. The immobilization of nitrogen is greatly influenced by the availability of the carbon sources within the pile for the duration of the three phases.

Carbon and nitrogen content in plant materials used for composting vary greatly. As plant changes during growth from young vegetative to maturation there is an inversion of the percent content of various components. Protein, nitrogen, and water soluble fractions decrease as hemicelluloses, cellulose and lignin increase. During decomposition of mature plants (Parr and Papendick, 1978) by microbes the readily

available fraction is used first (sugars, starch, organic acids, pecans, tannins, and nitrogen compounds). Reduction over time are the ether- and alcohol soluble fraction of fats, waxes, resins, oils, and the hemicelluloses and cellulose as they are used as a carbon and energy source. Lignin is overall resistant to decomposition (Moubasher, 1984) by microorganisms and is not greatly reduced over time. A study of two composts, one of wheat straw the other bean straw, demonstrated that over 251 days the lignin content was reduced by 0.35 and 0.52% respectively.

The availability and utilization of various compounds, either carbonaceous or nitrogenous, can change over time. A biochamber filled with winter wheat straw mixed with sand and water provided experimental results (Knapp, 1983) that demonstrate the respiration flux to response of biomass to additions of simple forms of both nitrogen and carbon during early stages of decomposition of wheat straw. In this study of 600 hours, it was shown that the availability of carbon is time dependent. Once the water soluble carbon was metabolized the system was carbon limited. The increase of nitrogen availability did not significantly increase the overall decomposition of wheat straw.

The ratio of carbon and nitrogen within the materials influence the microbial activity and the resulting biomass within the compost. Composting (Barrington, 2002) poultry manure mixed with corn cobs (C:N 20 or 25) resulted in losses of total nitrogen at 28 or 28% and carbon 32 or 23% respectively. In contrast, compost trials from the same study resulted in higher nitrogen losses with bulking agent with high percent of carbon that is resistant (lingo-cellulose) to microbial degradation. In a compost study using beef feedlot manure (Barrington, 2002) the nitrogen losses range from 19.3-61.5% of the initial nitrogen content. Of the attribute nitrogen losses 92% are the result of the

volatilization into the atmosphere in the form of ammonia and carbon losses in the form of CO_2 were between 46-62%.

Regardless of the substrate, the total amount will determine the maximum amount of growth for a particular group (Veldkamp, 1970). Within a specific amount of a substrate, one species will compete favorably with others (Foth, 1990; Veldkamp, 1970), that is some species are more efficient at nutrient utilization when offered lower amounts of substrate and will have a faster reproductive growth rate than that of a group that does better under conditions of high rates of the same amendment. In addition to this, composition of the material (Tuomela et al., 2000) will support only those able to make use of it as an energy source.

Substrate Particle Size

The particle size of the material affects the moisture retention, porosity, free air space, and the uniformity of decomposition in the end product (Day, 2001; Martin, 1992; Rynk and Richard, 2001). A compost with a high percentile of materials that are large will have an increased air space and the opposite will hold for materials that are small. Practitioners and researchers have established that it is optimum to have a mixture of materials that range in size from 3 to 55 mm. This provides for free air space of 32 to 36%. When the material size is large then shredding is done in conjunction with mixing. The increased surface area provides the microbes a larger number of sites for decomposition. The mixing decreases the potential carbon to nitrogen spatial differences and maximizes the porosity uniformity. Some operators opt to not shred the materials prior to mixing but wait until the end of the composting process to screen out the remaining large pieces. Primary materials for composting, such as sludge or slurry with

a high water content and primarily small particle size need to be bulked up, for example, with rice hulls or straw.

The composting process can be accelerated by decreasing the active phase to less than a week by using a rotating drum composter. This is a machine where the materials are fed (continuous or intermittent) into one end of the near horizontal drum and exit at the other end. With continuous or intermittent rotation of the drum, materials are mixed and exposed to ambient temperatures. The materials have increased exposure to high levels of oxygen and rapid dissipation of heat. One other method to reduce the active/hot composting process to less than 7 days (Biernbaum, unpublished data) is to decrease the particle size to finely ground material (straw, hay, and plant materials) passing through a 10 to 40 mesh screen and prepared in approximately 1 liter batches in a static but aerated and insulated container.

Moisture

The moisture content reported for aerobic composting is quite wide for what the general range and rather narrow for optimum. Moisture in compost comes from two sources: initial water in the feedstock materials and metabolic water produced by microbial metabolic activity (Stoffella and Kahn, 2001). Studies and reviews (Day, 2001) for a wide range of materials have placed the general moisture content to be somewhere at 25 to 80% or 25 to 60%. Other researchers are reported to establish the desired optimum range of 50-60% or specifically in the case of a particular mixture used in waste reduction to be 52 -58%. Research on the water content of the materials has focused predominately at the initial phase of composting.

In aerobic composting, microorganisms require that water be available to meet their metabolic and environmental needs. The percent availability of the total water from the material to meet the requirements is dependent on the fraction that is not tightly bound by material or salt. For microorganisms the water needs are met directly and indirectly from the material and water vapor. There is a selective process on the population if as a result of increased water potential the resource becomes limited. Authors (Bloom and Richard, 2002) in reviewing established constraints of microorganisms, cite several works in establishing that bacteria have a lower limit of -0.01 to -1.5 MPa of available water. Similar in size to bacteria and assumed similar constraints are: cocci, bacilli, spirilla, and actinomycetes. Fungi are reported to have the ability to function at water potential in a range of -0.01 and -10.0 MPa.

In contrast when there is an excess of water held within inter and intra matrix of materials the diffusion rate of oxygen to the sites with greatest potential difference is limited and under such conditions the metabolic processes governing decomposition are no longer aerobic.

In establishing the desired initial moisture content of material for composting the operator needs to calculate the water production and anticipated release. The metabolic processes of actively growing microorganisms resulting in an increase of biomass require water, energy (carbon), nutrient source (nitrogen), and in turn release of energy, carbon dioxide and water. In theoretical studies and experimental results (Day, 2001) the amount of water produced is 0.60-0.80 and 0.55-0.65 grams respectively for every 1.0 gram of organic material undergoing aerobic decomposition. In conjunction with this process there is the release/generation of 25 kJ of heat. This quantity of heat has the

capacity to volatize 10.2 grams of water. Therefore for every gram of organic matter decomposed aerobically, the maximum water produced is 0.80 grams and up to ten fold that amount potentially evaporated/volatized by generated heat. As temperatures increase so does the gas's capacity to retain water vapor. In addition to these dynamics as the internal temperature increases relative to the external temperatures there is a chimney effect that increases the rate of gas exchange resulting in an accelerated rate of vaporization. So in fact the required initial moisture content reported for composting is not needed for the process of decomposition but is required to account for loss by heat over time and is to ensure that there remain adequate amounts for the subsequent phases of decomposition and maturation.

Bloom and Richard (2002) conducted a study specifically looking into the constraints on microbes in the process of composting by the relative humidity and matric potential. The substrate had the moisture content reduced to a known value and the relative humidity was determined within the head space of a closed vessel. For compost with moisture content values above 25% the relative humidity consistently measured greater than 95% and those above 35% greater than 98%. Moisture sorption studies conducted (Barholomew and Norman, 1949) on various common plant materials demonstrate the relationship between moisture content equilibrium and relative humidity. The six plant materials were dried to approximately 8% moisture content and then exposed to an increased relative humidity from 15 to 100%. Between 15 and 20% there was little to no sorption. Starting at 20% relative humidity the net moisture content gain continued to increase geometrically (non- linearly) up to 95% and thereafter the moisture gain was only in relative small increments. The moisture content by sorption at

94.4% relative humidity for alfalfa, oat straw, pine needles, sudan grass, hemp bark, sudan grass root are 41, 30, 25, 35, 30, 20 percent respectively. The same materials exposed to a relative humidity of 100%, the moisture content reported is 52, 42, 30, 42, 39, 25 percent.

The relative humidity increase of 5.6% from 94.4 to 100% increases the moisture content of the respective materials by of 26, 40, 20, 20, 30, and 25%. Based on the data from these two reports, as long as there is sufficient water vapor for sorption by the material, high active water content is maintained by replacing water that became mobilized from the materials undergoing decomposition.

Aeration and Oxygen Concentration

The aeration and oxygen concentration within the materials undergoing decomposition effect microbial populations. If the decomposition of the materials is to remain an aerobic process (Parr and Papendick, 1978; Rynk and Richard, 2001) then the maintenance of the oxygen levels is critical. To monitor the changes occurring operators of composting facilities use an oxygen/carbon dioxide meter to determine the percent values of each gas. Typically the exit gasses are measured but it is recommended that the evaluation be done in situ as there are temporal/spatial differences within the mass.

Typically the oxygen levels are ambient just after the setting up the materials. This quickly changes and for a few days the levels remain around 10%. As the consumption of oxygen takes place the elevation of carbon dioxide is generally in a percentage ratio of 1:1. The rate of oxygen uptake is strongly correlated to the free air space in the pile. The rate of oxygen diffusion is greatly reduced in pore spaces filled with water. This limits the ability of the aerobic microbes to remain active (Henis,

1987a) but does not imply that their capacity to survive is impaired. It has been shown that the highest oxygen consumption rates are at low moisture levels where the water content is otherwise limiting. But this finding is not to be fast-held as in soil aggregates with intense microbial activity, anaerobic micro sites can result in those that are not water saturated. Sexstone et al. (1985) examined the oxygen concentrations within stable soil aggregates and found that the percent oxygen ranged from 21 to 0% over a seven millimeter distance. Three millimeters from the surface the oxygen level are below 5%. Under anaerobic conditions the mineralization efficiency and rate is considerably lower than that of aerobic conditions. A well aerated pile leads to the complete decomposition of any partially degraded metabolic byproducts. If the rate of gas exchange is limiting the ratio of aerobic: anaerobic processes becomes to narrow leading to incomplete degradation. The result of the anaerobic byproducts is offensive odors and the build up of phytotoxic compounds in the material.

Oxygen concentration levels in composting (Rynk, 1992) are said to be in a adequate range if greater than 5%. In reviewing the effects of gas concentration on aerobic microbes in soils (Parr and Papendick, 1978) oxygen become limiting at levels of 1–3%. The response to carbon dioxide varies greatly as some microbes responded favorably or adversely to high concentrations of the gas even under conditions with non limiting oxygen levels.

There a great number of ways to meet the aeration needs of the materials undergoing composting. Rynk (2001) reviews and describes in detail what is needed for each of the commercial systems that are currently used in the USA. Composting methods are characterized as either "out in the open composting method" or "in-vessel

or contained system". Under each of the systems there are five different sub methods as to how the materials are handled. The way aeration is achieved, in conjunction with the particular method is described as either passive, assisted passive, aerated, or forced aerated.

pH of the Feedstocks, Composting Mixture or Compost

In reviewing compost studies with agricultural wastes and food wastes, authors Day and Shaw (2001) report that as an overarching trend the pH of the decomposing materials in a compost initially undergoes a drop during the early phase. This is followed by an increase passing through a neutral pH and as the compost becomes a finished product it is slightly alkaline with values ranging from 7.5 to 8.5. Sullivan (2001) states that most compost finish with values between 6.0 and 8.0. Martin et al (1992) concur with these findings suggesting that the end product is best with values at or slightly higher than 7.0. The shift in values over the time of decomposition is initially the result of the production of acids which are later converted, changing the values to neutral to slightly alkaline, to carbon dioxide and methane. There are materials that strongly influence the final compost pH, for example wood tends to lead to an acidic product, whereas lime treated bio solids can be a sources of alkalinity.

In environmental studies on the effect of pH on the survival and dormancy of bacteria the distribution uniformity of the hydrogen ions is a critical factor (Henis, 1987a). Under acidic conditions the presence of clay mediates favorable conditions for bacterial populations at micro sites level. These sites provide a substantial number of bacteria survival opportunity to pass though the total sum change in pH from acidic to neutral values. Due to the heterotrophic nature of the microbial population (Day, 2001)

the process of composting is insensitive to a wide range of pH. That is to say that the materials will be decomposed by heterotrophic populations that are suited for the environmental conditions within the pile. This might give the impression that as pH changes there are large population shifts that occur. For some populations that may hold true but many bacteria (Atlas and Bartha, 1998) actually have a wide range of tolerance from 9.0 to an optimum growth at neutral and (Henis, 1987a) cannot grow beyond values lower than 5.5. Their capacity to adapt to such changing conditions is not well understood. The overall effect is that the bacterium cell walls resist degradation enabling the cytotoplasm to remain neutral so that normal functions take place.

Fungi on the other hand, have a wide range of pH conditions that they not only survive and develop under (Alexander, 1977). Dependent on carbon based materials as a nutrient resource they must have conditions that enable oxidative processes to take place. In environments where carbonaceous materials are or become available, different groups of fungi dominate the substrate as it goes through the decomposition. In settings with a low pH they tend to dominate. This is not the result of a preference for such conditions but rather that bacteria for the most part do not thrive at low pH.

Microbial Diversity of the Feedstocks and Composting Environment

The process of composting is primarily a biological process dominated by successions of microorganisms. The microorganisms come from the materials used for composting and in some cases cultures are added to the pile. Depending on the composting facility, one feedstock predominates over another. Materials used typically are plant based, animal body parts, or manure.

Primary plant material is colonized by bacteria and fungi while the plant is living. Once severed from the root transition colonization occurs towards decomposers. In contrast secondary plant material such as paper and cardboard has few microbial populations on its surfaces. Animal manure and human excrement have large microbial populations. Soil and mature compost are used at times, either as primary or secondary source of microbial populations. As a source of microbial populations if soil is used it is best selected where a highly diverse population is assumed to reside. Soil and/or mature compost can be mixed either uniformly throughout, placed in alternate layers, or as a single blanket layer over the pile.

The application of specifically cultured microbial populations to influence is done for example, to assure a beneficial population, increase resistance to a specific pathogen, or to facilitate the decomposition of a particular material within a system. This particular cultural activity with its specific goal is a practice done at the fewest of compositing facilities. The process of composting – the biological degradation of organic material – ending in the product compost does not depend on specific biological inoculum to be added.

Time or Stage of Process

The rate of progression from the initial setup through the thermophilic, mesophilic, and cooling phases is influenced by all the above variables as well as the site and management variables listed below. The types of organisms present, for example bacteria or fungi, will also be changing during the composting process. We would expect bacterial domination in early thermophilic stages with an addition increase of fungal species during cooling and maturation. To obtain compost with more consistent

microbial characteristics, one would have to depend more on the stage of composting and curing rather than a predetermined passing of a time interval unless, for example, the many variables are managed adequately to repeatedly provide the same product at any given point in time within the process of composting.

Site and Management

The site where the composting takes place influences the interface between the materials undergoing decomposition and the weather elements of temperature, water, wind, and soil. How these elements effect the decomposing materials are influenced by temporal and the geological location. For example the temperatures in the tropics and the temperate regions are sufficiently different to greatly influence the same materials. The four typical sites are an agricultural field, composting pad, covered bay, or fully enclosed each have distinct attributes which meet different needs of the managers (Rynk and Richard, 2001).

An agricultural field composting site is located near or in the field, typically the headlands, where the finished compost is to be applied. The elements are not mitigated and any changes to the pile after it has been set up are difficult. If water is needed to be added the transportation of water is often not feasible. To reduce water transpiration a fabric fleece can be used to increase the boundary layer and/or can also be applied during the curing phase to prevent the establishment of weeds. Without barriers regional air born (Hoitink, 1996) and soil organisms can colonize the pile during the entire process. Any disturbance imposed on the soil below typically has time to recoup before the next composting cycle occurs.

A composting pad is where the process is centrally located on a designated site. The base of the pad is made up of a clay/rock or asphalt/cement to accommodate heavy equipment and reduce ground water contamination. Water application in contrast to the previous site is much simplified by the reduced travel distance and stable driving surface. The use of a fleece meeting the same objectives can too be applied. The pad can be set with a gradient to prevent pooling of liquids which in turn are directed to a collection site.

A covered bay site offers protection from the rain and sun in addition to what a pad has to offer. Depending on the temporal/location at the time of set up there maybe either extreme of rainfall in addition to the drying effect of direct sun light on the pile. The equipment used at times is made up of fixed infrastructure such as slatted flooring, mixing machines, and watering systems.

A fully enclosed site provides for the highest degree of control of the weather elements. The reasons for such a facility may not be directly linked to the composting process but rather to contain environmental impacts such as dust, noise, and exit gasses to the neighboring properties. Concentration of airborne pollutants does adversely affect health of the operator and warrants that preventative safety measures are taken.

Compositing methods and systems

Once the materials are in place at the location where the actual composting process is to take place, specific management protocols are followed dependent on the method and system. Rynk and Richard (2001) outline the various manifestations of the combinations of method and system used into two general categories each with an additional five.

The first is referred to as the Open Method where materials undergoing decomposition are placed as freestanding windrows, piles, or open bins. Under the Open System the five categories are: 1) Turned Windrows in which the materials are organized as long narrow piles that are passively aerated and regularly turned. 2) Passively Aerated Static Piles are made of heaps that are for the most part not turned, but are infrequently in some cases. 3) Static Piles and Windrows with Assisted Passive Aeration where the material is not mixed but do have a simple system that delivers a higher surface area at the base of the pile, providing a greater potential for the passive movement of air to take place through the mass. 4) Aerated Static Piles and Bins this is much the same as the previous set up with the difference in that the circulation of air is forced through the materials. 5) Aerated and Turned Piles, Windrows, and Bins in which the materials are regularly or infrequently turned, have a system of aeration that is forced through the pile, windrow, or bins.

The second category is referred to as the In-Vessel or Contained Method where the composting take place in a vessel or a reactor with forced aeration and a means of controlling the environment around the material. Under this category the five categories are: 1) Horizontal Agitated Beds where composting takes place in long narrow beds with forced aeration where the agitation of the materials moves the mass progressively though the bins in a down stream manner; as new materials are added to one end the finished product comes out the other. 2) Aerated Containers where the enclosed materials have a system of forced aeration. 3) Aerated-Agitated Containers system is the same as the Horizontal Agitated Beds except that the process takes place in a commercial container. 4) Silo or Tower reactor in which the materials go vertically

through the reactor in a top to bottom manner and there is forced aeration. 5) Rotating Drums are slowly turning vessels where the materials enter one end as a fresh produce and exit the other as compost.

Intervention during the active phase can take place in the form a moisture management, product homogenization by mixing, and the flow of gasses through the mass. Some of the setups facilitate better the consolidation, though not a requirement, for the ease of handling the materials as the overall volume is reduced. The ability/necessity to intervene is method and system dependent in conjunction with the previously discussed factors that influence composting.

Intervention during the curing phase may take place. The nearly mature compost can be consolidated in to large piles or windrows with/out a type of fleece as a cover. Of primary importance is to ensure that before consolidation the materials are sufficiently decomposed so that reheating does not occur. Also important is that the moisture content be correct for the duration of the maturation phase so that neither saturation nor desiccation occurs. Under anaerobic phytotoxic compounds will build up or as the material dries more water sensitive microorganisms will become dormant or perish.

Factors Influencing Microbial Development in a Bioreactor

Bioreactors are used within laboratories and industrial complexes where microbes are maintained as cultures for better understanding by study or as part of a process in the production of compounds or food stuffs. The function of the bioreactor is to provide conditions as a framework in which controllable factors can be varied and the outcome observed or to produce a specific desired outcome. Overwhelmingly bioreactors use a liquid media for the production of cultures and products. The use of

bioreactors with solid state media (Whitney and Lynch, 1996) for the industrial fabrication of products via microbial synthesis is still in its infancy.

To better understand composting dynamics, one viable tool for study is to make use of a bioreactor where several factors are controllable. The study maybe set up to either emulate the composting process as found at a production site or to study the effects of controlled composting on a particular microbial population. The decomposition of organic materials within compost is in its nature a highly dynamic interplay of a multitude of variables equivalent to that of a disturbed environmental niche. A bio reactor facilitates understanding each of the attributing components insofar as the process is not so greatly altered (emancipated) away from the sequential process under study.

In studies of compost using reactors (Hansen, 1989) the factors that can be influenced or controlled are identified and characterized. The overall reactor dimensions, size, and distribution of the contents influence the effects of the other factors. The manner in which the contents are mixed before placement in the reactor influences the microbial dynamics in the reactor. In some cases the mixing process selected is one that replicates that which is found at a particular composting facility or perhaps uniformity is required throughout the material so it is homogenously mixed. The contents that will be used within the reactor are influenced by the material of study and the accompanying organic amendment. Once mixed their attributes of C:N ratio, particle size, and bulking agent will influence the microbial development. The microbial inoculums selected can come from laboratory cultures, soil, or previously made compost. It maybe that an inoculum is not used and the study depends on the resident populations of the materials.

A chemical pH moderation agent at times is selected to buffer or direct a selective process within the reactor. In the process of preparing the mixture the initial moisture content is a critical factor in providing water for the metabolic processes and influences the diffusion rate of gasses. As the study progresses the control of the moisture content maybe ether passive or active. Another factor is a stirring/mixing function and variable frequency so that all the materials are exposed to the internal locations within the rector. The flow rate, distribution and percent mix of oxygen are also variables that influence the populations within the reactor. Some reactors have a system whereby exit gasses are recycled through the material. Temperature within the reactor and its surroundings are of great importance. The flux can either be passive or controlled with systems designed to flush out or add heat to the system. The last is the retention and/or curing time of the product before extraction from the reactor.

In a study (Hansen, 1989) of composting of chicken manure, emulated in a bioreactor, seven of the controllable parameters were selected to understand their influence upon the rate at which composting took place. The parameters are the organic amendment, C:N ratio, mixing equipment, stirring frequency, initial moisture content, particle size, and ambient temperature. In order to evaluate the contribution of each factor, data taken from the biological activity was used. This included the changes in temperature, heat distribution, C/N ratio, mass, moisture, dry matter, and nitrogen. The greatest influence was found to be the type of amendment, mixing methods and initial dry solids' moisture content.

A study conducted to address a few of the needs of hypothesized long term space travel (Roberts, 2002) made use of a bioreactor to gain insight of the effects of a

controlled composting on a particular microbial population. In addition to understanding the carbon cycle the primary interest was the survival of human pathogens through the composting process within a bioreactor. The compost mixture comprised of field grown alfalfa hay, aspen wood shavings, and soil. The pathogens added to the mixture were 72 strains of E. coli and the same number of Salmonella strains from both environmental and clinical collections. The mixture and pathogens undergoing composting within the bioreactors were exposed to various temperature regimes and length of exposure time. The temperature settings were at either/alternating 25 or 55 °C in conjunction with different exposure time of either 3 and 9, 12 and 9, or singularly held at 12 days. The biological activity was monitored using the changes in media pH, C:N, dry weight, ash content, gas exchange of O_2 and CO_2, and the microbial communities by analyses of ergosterol and DNA.

Results of importance are the unanticipated extended lag time of anticipated changes (media, gas, microbial) in the experiments with the thermophilic temperature setting. The temperature had been imposed starting at time 0 upon the material, lead to the death or inability of mesophilic microorganisms to function and thereby excluded sequential precursors to environmental conditions for high temperature microflora. In this segment of the study temperature in conjunction with time, overrode the sequential processes of composting. In contrast materials without the elevated input of external heat sources demonstrated a shorter lag time and a rapid transition from mesophilic to thermophilic temperatures and accompanying organisms.

Proponents of the use aerated brewed compost tea call for the process to be done under aerobic conditions. For small scale production a bucket filled with water, perhaps

some amendments, inoculant from compost, and an aeration device in called for. Vessels larger than five gallons call for a device by the novel name of "a compost tea brewer" which is simply a bioreactor although they are not apparently viewed as such. Commercial manufactures have available sizes ranging from five to several thousands of gallons. To date there is a verification/certification system whereby the manufactures of the compost tea brewers are able to send in samples of aerated compost tea produced in their units and are evaluated against the benchmarks (set by the industry leader) of what constitutes a quality tea. Based on these results the production protocol is tweaked so that a consistent product can be produced and the unit is certified. Often the manufactures also have available amendments or "biological kits", with which their respective device produced the certified tested aerated brewed compost tea.

Disease suppression by application of compost or compost based products using a systematic approach

Litterrick et al. (2004) reviewed scientific journals (pier reviewed 1979-2003) for studies reporting suppression of disease as a result of treatment effect upon the interaction between plant and pathogen by the application of compost directly upon the soil (43 diseases 60 crops; 78 journals) or compost derived products applied to the foliage (20 diseases 25 crops; 29 journals). Publications authored out of the same institution in Ohio, USA (Al-Dahmani, 2003; Chung and Hoitink, 1990; Hoitink, 1996; Hoitink, 2001; Kuter, 1983; Zhang, 1998; Zhang, 1996) over an extended period has led to an evolving understanding of the effect of a particular compost has upon the pathogen plant interaction. The primary focuses of their research are commercial growers of green house crops grown in containers with soilles medium. The compost used is made of

easily accessible materials and a protocol to evaluate for its current efficacy to disrupt the disease cycle can be established. The duration of its efficacy is correlated to the remaining available carbon. The mode of action that interrupts the disease cycle is proposed to be a primaraly a biological one. Autoclaved compost did not suppress disease but when it was reinoculated with either compost or bacterial cultures it regained the desired suppressive attributes. Publications authored out of the same institution in Wisconsin, USA (Cronin, 1996; Yohalem, 1996; Yohalem, 1994) reported the effect of using specific compost derived water extract upon the germination of pathogen conidia in vitro, disease development on green house seedlings and trees in the orchard. The effective inhibition of the germination of conidia *Venturia inaequalis* in vitro test and the resulting disease, apple scab, on green house apple seedlings was reported. During the concurrent year no differances from the water control was established in the orchard apple trees. Also screened were pathogen conidia in vitro and seedling assays with their respective disease for maize, red pine, and ginseng. Promising results as a basis for further research was reported for the associated conidia and disease development on the maize and red pine. Using the same protocol for production of the compost derived water extract, in a later study over three consecutive growing seasons, apple trees in two different orchards susceptible to apple scab were evaluated for the treatment effect of the foliar application. The extracts significantly reduced both the leaf area affected and the disease incidence better than the negative water control but not as well as the conventional chemical compound Captan. The compost used was spent mushroom substrate provided by three commercial mushroom producers using similar production protocols. The compost was mixed with water (1:2 wt/vol compost/water) and left at

room temperature to ferment under non aerated conditions. In the first series of experiments the reported 6-9 days of extraction in water provided the most effective conidia germination inhibition, and seedling disease control. The product remained stable and effective under three storage conditions (-20, 4 °C and room temperature) for four months. The authors concluded that the primary mode of action within the extract to be heat stable compound as autoclaving or filter sterilization did not change its efficacy. There were significant differances in the efficacy of the extract made from the spent mushroom substrate from the different producers.

Both biocontrol agents and pathogens as saprophytes invade organic materials that have readily available cellulose. During various stages of composting materials there can be significant amounts of cellulose available for both types of saprophytes. In reviewing studies with the disease suppressive qualities of compost, Hoitink et al. (2001) concludes that the composted material must have undergone sufficient decomposition as to not have any phytotoxic effects and have been colonized by microorganisms that are to provide the biological control between a specific pathogen and host. The form of disease suppression achieved by compost that has the desired biocontrol agents are described as either general or specific. General suppression is described as the overall reduction of the capacity of the pathogens to grow in the presence of compost. That is to say that a wide range of pathogens are unable to germinate, invade and proliferate in or near plant tissue in the presence of compost. The concept of specific suppression is based on the fact that some pathogens are directly controlled only by a few specific agents. If the intended agent in not actively present in the compost the suppression to the specific target organism will not occur. But this does not preclude the possibility of an

effective mode of general suppression. Once the desired suppressive qualities are achieved they are not permanently maintained. As the decomposition of the material progresses over time it no longer provides support to the bio controlling mechanism. With the expectations of a few documents (Hoitink, 1996), detailed guide lines ensuring the disease specific suppressive qualities of compost are not available.

In addition to the more direct nature of action between the biocontrol and pathogen agents it is reported that there is a response by plants growing with part of their roots in direct contact with compost. A study (Zhang, 1996) using cucumber plants grown in potting media with half of the root system in compost, the other in peat, had the entire root system resistant to root rot. The contact by the root system to the contents of the compost, it appears, does not elicit a full systemic response until after the infection by the pathogen. In effect the plant is better prepared to respond to an active assault and provides insight that the desired results may not only be primarily located in the media material (field or container grown) but also may have a secondary systemic mode of action.

Hoitink et al. (2001) presents a sequence of events and conditions that are required for the production of such compost from pine bark for use in container grown plants. It is during the second phase (55 to 70 °C) of composting, during which seeds and pathogens are destroyed, so too are the beneficial microorganisms that provide control of over pathogens. As mesophiles, the biocontrol agents are described as recolonizing the materials as temperature decline and remain below 40 °C. The colonization is to occur from the peripheral surfaces and progresses inward to the center of the pile. It is during the curing phase of more than four months that the level of

biocontrol is achieved. In addition to temperature, it is during the curing phase that conditions with a moister content of 40 to 50% and a pH greater than 5.0 are conducive to the control agents.

Zhang et al. (1998) used a compost water extract applied to the foliage on cucumber and Arabidopsis plants to evaluate plant response to pathogen application in conjunction with plants grown ether on peat, compost based, autoclaved compost based media or reinoclated by with the non-autoclaved compost or a pure bacterial population isolated and cultured from starting compost. The compost water extract was prepared by placing water and compost (1:1, vol/vol) at room temperature to ferment for seven days and then filtered with cheese cloth and paper filter. Additional solutions from the compost water extract sterilized by filtration or autoclaving and there from some reinoculated with the non-autoclaved preparation or by a pure bacterial population isolated and cultured from starting material. Extracts, negative, and positive control, were sprayed on the foliage at full strength at seven and two days before the plants were tested for treatment effect in growth chambers by exposure to the disease vector. The cucumber grown in compost based media or reinoculated with non-autoclaved compost were found to have induced systemic resistance. Plants grown in autoclaved compost mix and a few of the reinoculated mixes by pure culture did not elicit induced systemic resistance. The authors concluded that the mode of action is biological in nature and had up until publication not able to isolate the responsible microbes. Arabidopsis grown on peat with the applications of compost water extract had disease indices similar to that of the positive control. The authors concluded that the mode of action to be biological in

nature as the autoclaved or filter sterilized extracts did not provide the reduction of the disease.

The reduction of disease was reported in the examples above using aerobic or anaerobic protocols involving compost or compost based products as either rooting media or as foliar spray application. Authors concluded that the primary mode of action from their experiments to be abiotic or biotic. This is an indication the application of compost or derivatives thereof may promote plant health in different ways. Regardless of the mode of action the research leading an understanding of the suppressive nature of the compost or compost based products are attained by using a systematic approach to the production protocol and evaluation methodology.

Summary

There are several variables in regards to compost and the composting process as well as compost tea and the tea making process. However, the number of variables and the nature of the variables are manageable. It appears to be feasible to make repeatable batches of compost with specific characteristics. The tea making process can also be managed. However, it appears that managing the variables in a solid state such as in the composting process is easier on a small scale or on farm scale than trying to manage variables such as temperature, aeration and substrate availability in a liquid culture medium.

In chapters 2 and 3 of this thesis, methods to avoid the cultivation of microbes in the solution phase are outlined. Rather than cultivating microbes in a liquid system for production of compost tea, it is recommended to manage microbial growth in the solid phase compost by adding an appropriate substrate to mature, cured compost under

managed moisture, aeration and temperature conditions. The biologically activated compost is then quickly extracted with water to provide a compost extract with all the desired characteristics of previously reported compost tea. The extracts however are viewed more favorable under the current set of regulations and are much easier to prepare on farm and in small batches.

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Chapter II

Incubation of Mature Compost Substrate and Alfalfa Based Amendment for Production of Activated Compost Water Extract: Three Preliminary Experiments

Abstract

The addition of organic material such as uncomposted vegetative matter, compost, manure, and extracts of compost or manure have been used in agricultural systems to improve soil and plant health (Litterrick, 2004). A bioreactor was developed to cultivate microbial populations in a solid state media under controlled conditions for the eventual extraction into water and application to plants. A mature compost substrate was used as the medium within the bioreactor and incubated with or without an alfalfa based amendment under conditions of either low temperature and high oxygen, low temperature and low oxygen, or high temperature and low oxygen. The temperature within the bioreactor can be regulated and the environmentally correct thermal succession is regulated in conjunction with a "guide" bioreactor. The "guide" bioreactor does not have its temperature regulated as internal temperatures rise as the result of exothermic processes taking place within the medium. Once the target temperature of the treatment bioreactors is reached it is maintained. The incoming gas O_2 percentage was regulated to have a maximum value (3% or 21%) at a constant flow rate. Temperature, CO_2 and O_2%, nematode and bacterial populations, moisture content, pH, and electrical conductivity (EC) were measured. The CO_2 and O_2 percent and percent gradient, and grams of CO_2 released for the projected maximum and hour of 1/2 maximum were calculated. Substrate pH ranged from 6.36 to 8.9 which are within the anticipated norms of composting. In both amended and non-amended media the moisture content was maintained between 36% and 41% (mean 38%). Under low temperatures with an inflow of 21% O_2, outflow remained above 13.89%. With 3%

inflow oxygen under low and high temperatures outflow ranged from 0.54% to 2.79% (25 °C) and 0.24% to 2.97% (40 °C). The total projected grams of CO_2 released and the hour at which 1/2 maximum occurred for the three treatments ranged from 11.92 to 17.12 g and 35.35 to 65.45 h. The nematode population (bacterial feeding) within the amended media significantly increased over time under both oxygen treatments at 25 °C (not evaluated at 40 °C). The total bacterial population numbers (treatment of elevated temperature and low oxygen) appear to both lag and not increase in "tandem" with O_2 reductions and CO_2 increase.

Introduction

Composting involves the collection and placement of organic materials amassed in a pile or within a constructed bunker where the material can be manipulated, and there by influencing the process, by mixing, aeration, moisture, and temperature management regulation. Of importance is that exothermal heat generates temperature in the range of 45 to 70 °C so that there is a reduction of viable seeds and resident plant, human or animal pathogens. Over the course of time the material will change in volume, texture, appearance, odor, stability, and resident microbial population. Typically composting is done on an agricultural field, composting pad, covered bay, or fully enclosed site which all influence the interface between the compost and weather elements of temperature, water, wind, and sun. Rynk and Richard (2001) outline two general methods and systems used for composting. The first being the Open Method where materials are placed as freestanding windrows, piles, or open bins, and the second is the In-Vessel or Contained Method where composting takes place in a vessel or a bioreactor with forced

aeration and a means of controlling the environment around the material. The Environmental Protection Agency and United States Department of Agriculture (2005) have published guidelines and requirements defining the process of composting and the product compost. The United States Department of Agriculture has specifically addressed the definitions and use of compost in organic agriculture as outlined by the National Organic Program (NOP) (2000).

Compost is mature when it suitable for a specific use and its stability is the degree of resistance to further degradation over time (Sullivan and Miller, 2001). The use of compost as an amendment to cultivated soils as part of a fertility program (Stoffella and Kahn, 2001) leads to a reduced bulk density, an increase in organic mater, water retention, microbial diversity, and long term fertility. In organic farming this amendment is an integral part of both the soil fertility and disease/insect management plans for improved plant health. Litterrick et al. (2004) identified 78 peer reviewed publications (1979 to 2003) of pathogen suppression as a result of amended soils for 43 different diseases on 60 crops. Currently the descriptive mechanisms Litterrick et al., (2004) of disease suppression are 1) induced protection, 2) hyperparasitism, 3) antibiosis, and 4) competition for nutrients. Protocols have been developed (Hoitink, 1996; Hoitink, 2001) for predicating the suppressive attributes of a specific compost for a few select pathogens. The suppressive attributes are attributed to mesophilic microorganisims are described as recolonizing the materials as temperature decline and remain below 40 °C. The colonization is to occur from the peripheral surfaces and progresses inward to the center of the pile. It is during the curing phase of more than four months that the desired level of biocontrol is achieved. In addition to temperature, a

moister content of 40% to 50% and a pH greater than 5.0 are conducive for the development of the suppressive qualities. The product compost is considered fit for use when requirements set by the end user are met. Sullivan and Miller (2001) summarized a large number of methods and protocols developed to evaluate the compost. Five overarching quality indicators include that the process of composting and the product compost with the intended application to promote plant health are : 1) a thermophilic phase for significant reduction of weed seed and pathogens, 2) not undergone prolonged anaerobic conditions, 3) self heating no longer occurs (not simply due to a lack of moisture), 4) during the curing phase a moister content that did not restrict gas exchange promoting the development of anaerobic microbes and compounds, and 5) contains a large and diverse population of desirable microorganisms.

Compost tea is defined as a filtered product that is manufactured by placing compost in water under either aerobic or anaerobic conditions that has undergone fermentative processes. Compost extract is a filtered suspension of compost and water that has not been fermented. In a review of compost tea and extract of 29 peer reviewed publications (1988 to 2003) Litterrick et al. (2004) identified examples of proven disease suppression of 20 pathogens on 25 crops by use of primarily of anaerobic compost tea. Over the past few years the production protocol favored in the USA is that of an aerated compost tea avoiding phytotoxic metabolites and associated odors. Appropriate Technology Transfer for Rural Areas (ATTRA) (2005) of the National Sustainable Agriculture Information Service provides current production methods, application, and regulations. There does not appear to be evidence to support the importance of aeration and the required expenses of systems designed to provide the aeration.

Recommendations by Ingham (2003), seen currently in the USA as an industry leader, for production and application aerated "Brewed Compost Tea" are as follows: Compost is placed in a container that is suspended in a brew vessel filled with an aerated nutrient-water solution. The recommended brew time is to be optimum between 12 to 48 h based on the microbial diversity and total biomass of organisms (no data given). The compost water ratio (vol/vol) most commonly used is 1:10 but can range from 0.3:10 to 2.5:10. The compost tea is descried to be either bacterial or fungal and depending on the desired type of microbial growth the recommended energy source used may be a prepared nutrient/biological mixture, sugar, plant extracts, fatty acid, cellulose waxes, or humic acid. The water used must not contain high levels of salts, nitrate, heavy metals, sulfur, tannic acid, carbonates or disinfectant materials and is warmed within one and a half degrees of 20 °C. Within the brew vessel, regardless of model, emphasis is placed on maintaining dissolved oxygen levels greater than 5.5 ppm. The production location is to buffer direct sunlight and temperature fluctuations. The coarsely filtered end product is applied either at full strength or diluted and post production products may also be added such as surfactants, stickers, or nutritive products. The product is applied with a low pressure delivery device as a drench or spray to either the soil or the aerial structure of plants. There are few analytical labs in the USA that specialize in evaluation or characterization of compost tea. Interpretative evaluations are done based on a proprietary data base and recommendations are made for agricultural use. The National Organic Standards Board Compost Tea Task Force (2004) addressed a wide range of concerns in the application of compost extracts or ferments on crops intended for human consumption. The difference between compost

tea and compost extract was defined as: "...any mixture of compost and water that is held for longer than one hour before initiating application to be a form of compost tea. Any mixture of compost and water that is held for less than one hour before initiation application is considered a compost extract".

Factors that influence microbial development in compost that can be regulated are temperature, moisture, the substrate/feedstock C:N ratio, particle size and, spatial distribution, aeration, pH, microbes present or introduced, and the compositing site and pile management. Typical ranges over the course of time are maximum temperatures of 48 to 71 °C, initial 30% to 60% moisture content, initial C:N of 40:1 to 25:1, a mixture of particle size ranging from 0.3 to 0.55 cm providing respectively increased surface aria for microbial activity and increased structure and porosity, oxygen levels of internal macro pores ranging from 2% to 21%, and a flux of pH ranging from 6.0 to 8.0. The temperature over the course of the process is greatly influenced by pile size, moisture content, porosity, and available energy.

The function of a bioreactor is to provide conditions as a framework in which controllable factors can be varied and the outcome observed or to produce a specific desired outcome. The use of bioreactors with solid state media (Whitney and Lynch, 1996) is still in its infancy. In studies of compost using bioreactors (Hansen, 1989) the factors that can be influenced or controlled are the same as those identified for composting plus the bioreactor dimensions, size, and the manner in with the contents are mixed and distributed. A bioreactor facilitates understanding each of the attributing variables insofar as the process is not so greatly altered (emancipated) from the normal sequential process of composting.

While our initial goal was to test the efficacy of compost tea on apple scab in an organic orchard, it became clear that there was not a standard tea production method that would easily and consistently produce an expanded range of product for testing. The advantage of water based systems with heavy aeration is that the microbial population and any water soluble abiotic compounds are retained in solution ready for application in 24 to 48 h. The primary means recommended directing the development of the biological process is limited to the type and quantity of amendment used. The type of amendment used is, according to recommendations (Ingham,2003), will produce a product with either a bacterial or fungal emphasis. The maximum quantity of amendment used is defined by maintaining the O_2 saturation above 6%. In addition this production system provides limited ability to alter temperature and oxygen supply. Current recommendations restrict (limit) the use of manure based compost and activating amendments due to concerns of contamination with human pathogenic *E. coli* (O157:H7) under anaerobic fermentation conditions.

In preliminary studies it was observed that the amount of amendment mixed with the mature compost had an effect on the self heating processes and nematode populations within the solid state incubation vessel. It was established in a previous study (unpublished) under similar conditions that 97% of the nematodes accounted for the overall population increase were bacterial feeders. This group of nematodes has a minimum reproductive cycle of approximately 72 h. The energy required for such an increase can only come from a surge of prey for this organism. These studies also identified problems with substrate moisture stratification and regulation in the incubation vessel with and without continuous gas flow through the media.

Based on our independent observations of a) apparent rapid microbial growth in compost based transplant media following incorporation of organic fertilizers (soybean or alfalfa based), b) the identification of diverse nematode populations based on feeding function (bacterial, fungal, omnivore, and carnivorous) in mature composts, and c) the knowledge that cooked rice is often used as a substrate for fungal cultures, we propose to test a method of producing activated compost water extracts. A bioreactor developed over previous studies will be tested by placing non-amended and amended mature compost under various treatment conditions. Replicate bioreactors will be monitored by measuring temperature and respiration rates. This and additional data from tests preformed directly on the treated substrate, will also provide insight to the microbial response to treatments of managed moisture, oxygen, and temperature in a solid state substrate bioreactor. Provided that results demonstrate reliability of the bioreactor and changes substantiated of the microbial population, then the methodology for the production of activated compost water extract will be had so that the treated substrate can be used for a rapid (20 minutes) extraction with water. This production of compost extract will provide two distinct advantages with the capability for modulation of controllable variables and avoids the limitations and food safety concerns associated with compost tea.

Methods

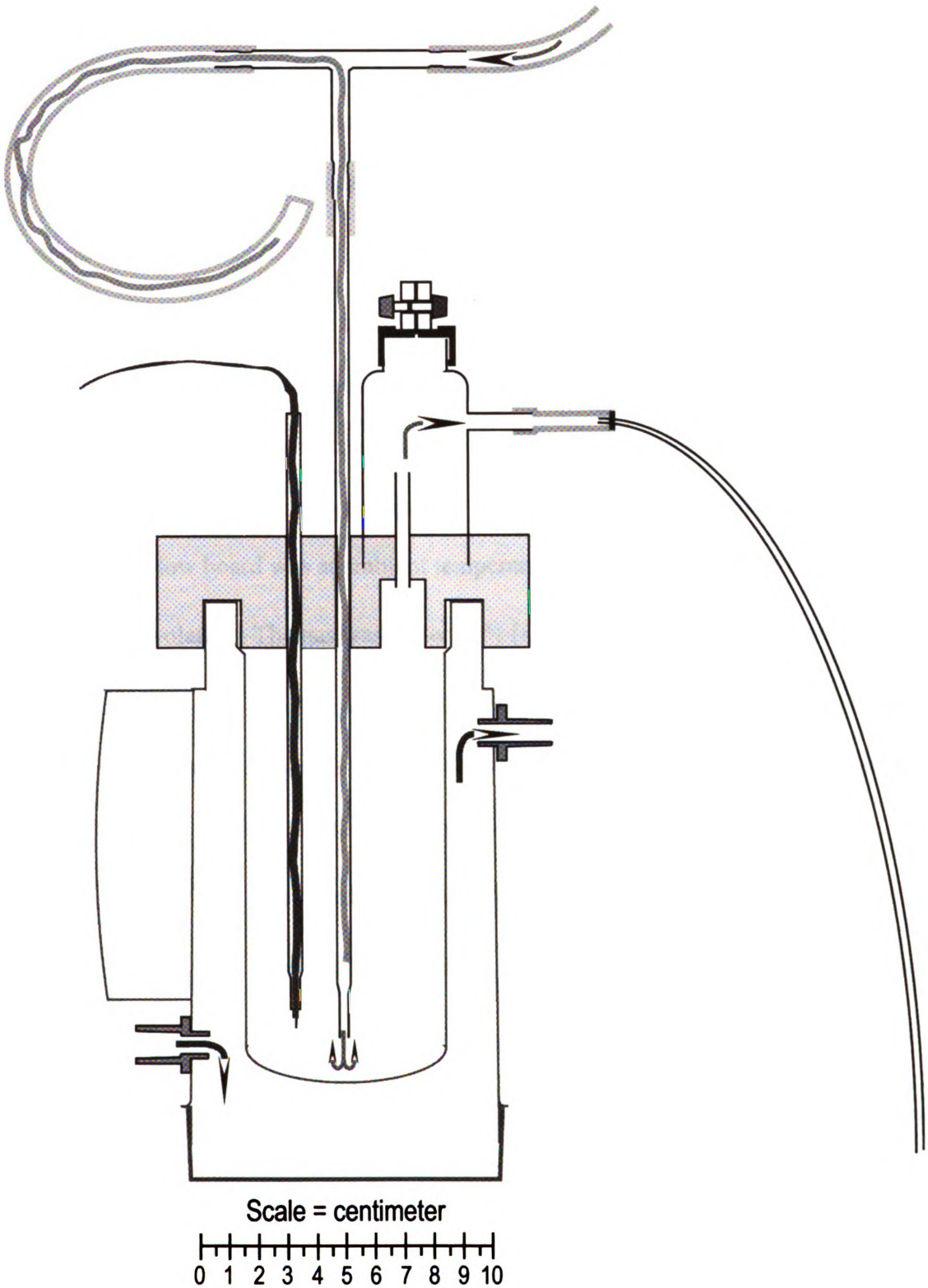
A bioreactor was made from a modified glass insulated wide mouth soup Thermos™ jug with an internal volume of 0.5 L with a constructed polystyrene platform lid to support hardware. The hardware was used to monitor temperature, percent O_2 and

CO_2, and deliver a continuous moisture regulated gas flow through the medium. The moisture content of the amended medium in pilot studies using bioreactor prototypes resulted in either horizontal migration of moisture from the one section to another resulting in distinct moisture zones or complete drying out of a 2 cm^3 zone around the orifice of the inflowing gas. The current device is to address these trends of moisture shifts without substantially altering the initial moisture content by automatically increasing the relative humidity of the incoming gas in conjunction with changing temperature. A significant contribution is that relatively low media moisture content can be used and results in an increased porosity and surface area for gas exchange. The jug was modified so the insulating material around the liner was temperature controlled by recirculating water (Figure 2.1). (See Appendix A for a detailed description of the construction, components and setup protocol of the bioreactor as it evolved through twelve studies.)

For this study a compost was specifically made from bulk soft wood shavings, baled straw, sphagnum peat, fresh harvested comfrey foliage, soil, blend grass hay, and baled alfalfa hay (approximately equal parts by volume) maintained out of doors on soil and passively aerated. The mature compost was screened through a hardware cloth (0.6 x 0.6 cm) to remove macro pieces of organic material and used in the bioreactor without or with an amendment. The amendment used, an alfalfa based organic fertilizer (Bradfield Organics®; Alfalfa (3-1-5), sulfate of potash, molasses, poultry protein, humate, C:N 1:13, ash 15%), was ground and screened (< 0.2 cm) before being mixed at a rate of 23.6 g for every 0.5 L compost. The moisture content of the compost without or with amendment was increased to 37% with RO water. In the bioreactor the density and

porosity of non-amended compost was 303 $kg \cdot m^{-3}$ and 0.471 $m^{-3} \cdot m^{-3}$ and with amendment 307 $kg \cdot m^{-3}$ and 0.466 $m^{-3} \cdot m^{-3}$.

Figure 2.1. Scale drawing of the solid state bioreactor.


Scale = centimeter
0 1 2 3 4 5 6 7 8 9 10

For the remainder of the text, as of time zero the non-amended and amended mature compost, that is to or has undergone treatment in a bioreactor, will be referred to as the non-amended or amended medium.

The medium was maintained with a continuous flow of gas regulated by individual capillary tubes (52 mL (+/-3%) · minute $^{-1}$) 1.5 cm off the bottom and laterally centered within the chamber. The rate was determined by a previous study to evaluate various flow rates and reduction of oxygen through the same medium. The gas was distributed by a flow board constructed of capillary tubes under constant gaseous pressure, regulated by a single stage regulator in conjunction with a bubble meter. The gas exiting the flow board was at ambient temperature and scrubbed to remove any compressor oil volatiles. The gas was either 21% (ambient) or 3% O_2 by using two gas regulators and a mixing chamber to combine atmospheric air and di-nitrogen gas. Over the duration of the treatments using reduced oxygen levels, samples were taken after the mixing chamber to confirm that the target of 3% was maintained.

Respiration was calculated from the percent CO_2 production and O_2 depletion by using a 0.1 to 0.5 mL gas sample (infrared gas analyzer Model 225-MK3, Analytical Dev. Co., Hoddesdon, United Kingdom and paramagnetic O_2 analyzer Servomex series 1100, Servomex Co., Sussex, England connected in series with N_2 as a carrier gas with a flow rate of 100 mL·min^{-1}). A certified gas standard (Matheson Gas Products, Chicago, IL) and atmospheric air (O_2 only) were used to calculate respiratory gas concentrations of three samples per bioreactor per time point. For samples taken from bioreactors

containing non-amended media the relative humidity of the atmospheric standard was taken into consideration based on local meteorological data from which the actual percentage of oxygen (Y= -0.00049x + 20.946) was obtained. The data collected from bioreactors with amended media were transformed to represent the gas percent gradient and processed using software program Table Curve 2D v5.01 (SYSTAT, Software Inc.). An equation selected from the software library fit the data well and provide as constants the hour and height of the peak values and allowed calculations of the total respiration. For those bioreactors filled with non-amended media the percent gradient was calculated based upon the slope of a best fit line of the data using Excel™. In the interest of quantifying the respiration capacity by the microbial population of amended medium, the maximum grams of CO_2 released for each treatment was determined by extending the curve until the state of equilibrium was reached. Based upon the hour and total sum value at equilibrium, the hour at which half of the maximum CO_2 was released was calculated. When calculating the CO_2 values of the amended medium gradient, one of the variables of the equation based on the non-amended medium was modified to withdraw the background CO_2 generated. An additional curve was generated for a second accumulative grams respiration for CO_2 based upon the O_2 respiration gradient equation.

In an experiment (Roberts, 2002) using solid state bioreactors with thermophilic temperature (55 °C) setting imposed at time zero, an unanticipated extended lag time of anticipated changes (media, gas, microbial) occurred. The proposed explanation was the death or inability of mesophilic microorganisms to function and thereby the failure to

form excluded sequential precursors necessary for high temperature microflora. In order to regulate the temperature within the experimental bioreactors, a "guide" bioreactor with the original insulating glass vacuum liner of the unaltered Thermos™ was used to emulate the natural temperature progression provided by the mass of a larger composting process. The temperature rise and transition from ambient to elevated temperatures as a result of self heating was monitored. As incremental increases were detected the corresponding temperature was increased for the experimental bioreactors by the water regulating system surrounding the mixture. Once the designated target temperature was reached (25 or 40 °C) it was maintained for the duration of the study. Thermocouples placed within the media of each bioreactor were monitored with either a hand held device or data logger.

The three separate experiments took place in a convection walk-in-cooler maintained at 22 °C for the duration of the study. Three replicate samples were taken from the non-amended and amended medium at time zero. Depending on the specific experiment the four or five individual bioreactors harvested at the same time constituted replicates.

At the designated harvest point the bioreactor contents were emptied into a plastic bag and thoroughly mixed before aliquots were taken for testing. Medium pH and electrical conductivity (EC) were determined using volumetric dilution of 1:1 and 1:2 respectively. The moisture content was determined from a sample dried in a convection drying oven at 110 °C. In two of the three experiments the nematode population was evaluated in a 100 cm^3 sample by sugar flotation and centrifugation protocol (Jenkins, 1964) as used by MSU diagnostics lab. A light microscope was used for a direct

population count and the average value is derived from four samples taken from the starting media, bulk mixture at time zero, or from each of the bioreactors within a harvest interval. When densities were too numerous a dilution was made and there from four aliquots enumerated. Standard analysis of variance and means separation of medium pH, EC, moisture content, and nematode population were by SAS LS means.

Results

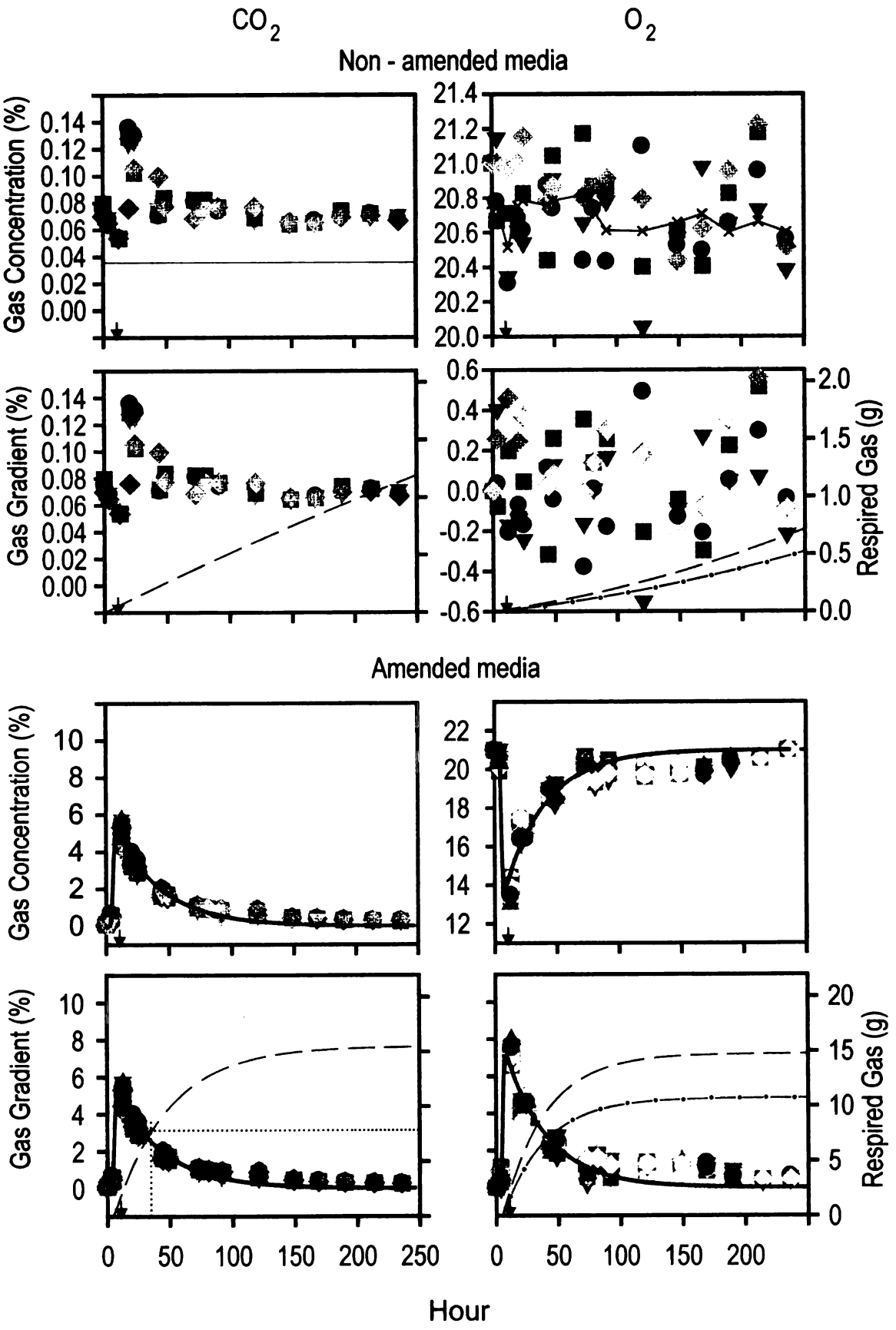
Experiment 1: High O_2, Low temperature

Specific to this experimental in addition to the general methods described twenty four bioreactors were prepared and designated into one of six sets (Sets 1 to 6) of four. The gas entering was atmospheric. Set 1 to 5 all had the same amended medium and Set 6 had non-amended medium. The "guide" reached the target temperature after 10.25 h at which point the water regulating system was set at 25 °C (Figure 2.2). The confirmed internal media temperature range was 23.0 to 25.5 °C for remainder of the experiment.

For non-amended media the O_2 and CO_2 levels exiting the bioreactors were determined to be between 20.05% to 21.22% and 0.05% to 0.14% respectively. Data from the bioreactors with the amended medium fitted with an equation (#8062 Table Curve 2D v5.01) SYSTAT, Software Inc.). determined the lowest O_2 level was 13.88% at 8 h and CO_2 levels peaked at 8.75 h with a value of 5.03% (Figure 2.2). The gradient (%) for both CO_2 and O_2 of the non-amended and amended medium treatments are in

Figure 2.2. The potential mass of CO_2 respired by the amended media was calculated to be 15.50 g with half of the maximum CO_2 respired at 35.4 h (Figure 2.2 Table 2.1).

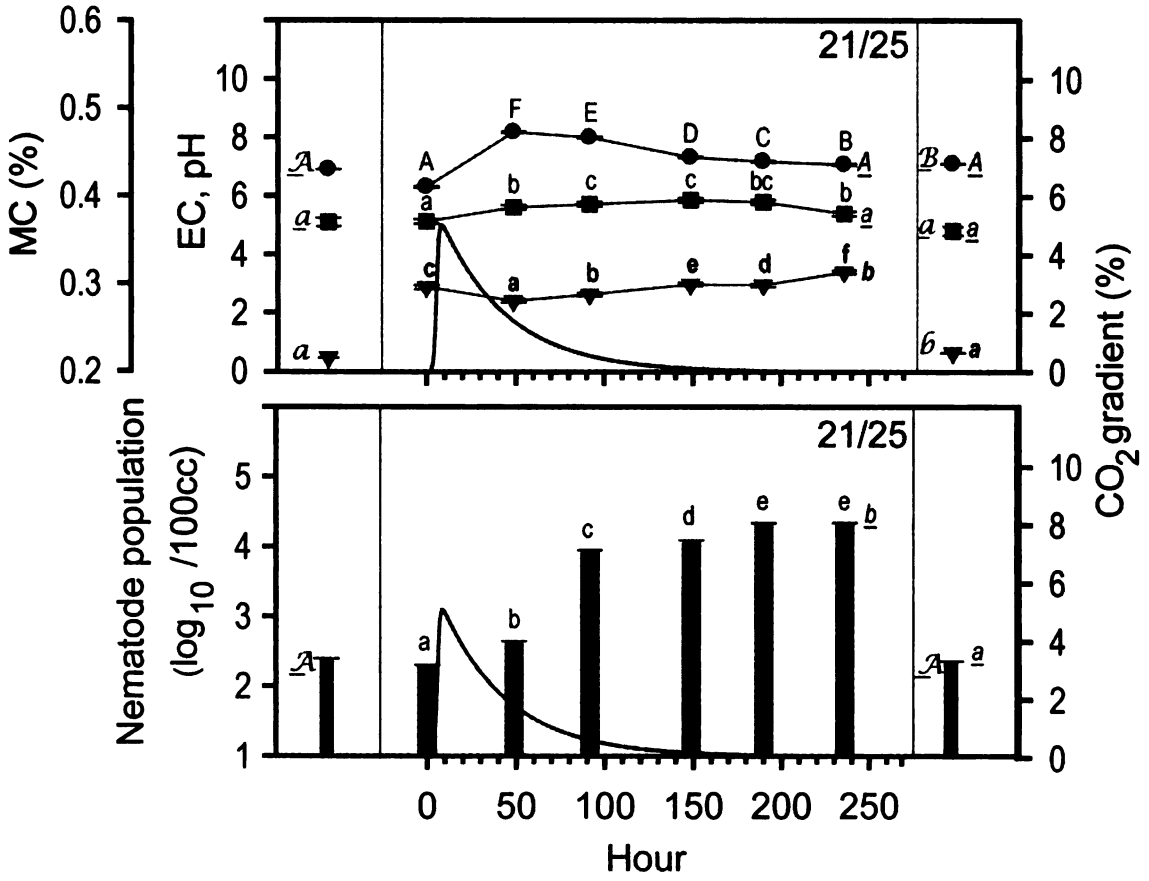
Figure 2.2. Gas concentration (%), gas gradient (%) and respired respiratory gas (g) of non-amended or amended medium under treatment of 21% O_2 influx and 25 °C. Solid fine line is the given atmospheric CO_2%. The solid line interspersed with Xs is the adjusted atmospheric O_2% flowing into media. Arrow indicates hour at which the target internal temperature was reached by self-heating (amended) at which point it is maintained at target value. For bioreactors filled with non-amended media the respired respiratory gas (g) was calculated based upon the slope of a best fit line (not shown) of the data using Excel™. The dashed line with dots is the accumulated consumption of oxygen based on the best fit line. Long dashed line is the accumulated release of carbon dioxide (g) based on either the accumulative sum of the CO_2 or O_2 gas gradient (%) equation. For bioreactors filled with amended media the bold solid line is the fitted equation (#8062 Table Curve 2D v5.01 SYSTAT, Software Inc.) based on sample data. The dashed line with dots is the accumulated consumption of oxygen (g) based on the O_2 gas gradient (%) equation from the media. Long dashed line is the accumulated release of carbon dioxide (g) based on either the accumulative sum of the CO_2 or O_2 gas gradient (%) equation. The fine doted line indicates the hour and value (g) at which half of the maximum CO_2 respiration was reached.


CO_2
O_2
Non - amended media
Gas Concentration (%)
Gas Gradient (%)
Respired Gas (g)
Amended media
Hour

The electrical conductivity (EC) of the non-amended and amended media at the initial mix respectively was 0.62 and 2.90 $dS \cdot m^{-1}$. Over the experiment duration the trend in EC change was for an initial decrease then increase which is representative of the composting process. The biological significance of these changes are not expected to significantly influence microbial populations. The initial change is attributed primarily to the K_2SO_4 from the alfalfa based fertilizer used as the amendment is calculated to have added approximately 800 ppm soluble salts to the system. The amended medium had an initial pH of 6.33 that increased to the highest value of 8.20 by 48.75 h and gradually decreased to 7.1 at 236 h which is representative of the composting processes and is attributed to the process of mineralization of the amendment. The non-amended medium moisture content did not change after 236 h of treatment. There were no differences between the non-amended and amended medium after 236 h of treatment. The amended medium moisture content over the duration of treatment did undergo changes with a range of 37.2% to 39.6%, which is not expected to influence the microbial population or be biologically significant (Figure 2.3). The nematode population density ($\log_{10} \cdot 100\ cm^{-3}$) of non-amended media (2.3) did not change while amended media underwent significant changes over the duration of 236 h. The amended medium from time zero through 190 h the population increase was from 2.27 to 2.62, 3.92, 4.06 and 4.30 respectively (Figure 2.3). The actual average nematode population extracted from the media is note worthy. The non-amended and amended medium had the respective values of 236 and 188 at time zero. At 48.75 h the amended medium contained a population of 432 which is a 2.3 fold increase. The population continued to

increase with 8314 (91.75 h), 11417 (148.75 h), 20184 (190.10 h), and 20509 (236.00 h) which represents a 44.2, 61.7, 107.4, and 109.1 fold increase over that of the starting mix. On 360 h, the non-amended media that underwent treatment within the bioreactor had an average value of 215 which is biologically negligible with that of the starting non-amended and amended medium at time zero.

Figure 2.3. EC ($dS \cdot m^{-1}$), pH, moisture content, and nematode population (circle, square, triangle, bar) of non-amended and amended medium under treatment of 21% O_2 influx and 25 °C. Font size and style designate the statistical evaluations done. Graph segment without designated x axis units is that of the non-amended medium before and after treatment. The solid line represents the CO_2 gas gradient (%).


MC (%)
EC, pH
CO_2 gradient (%)
21/25
21/25
Nematode population
(log_{10} /100cc)
Hour
0
50
100
150
200
250

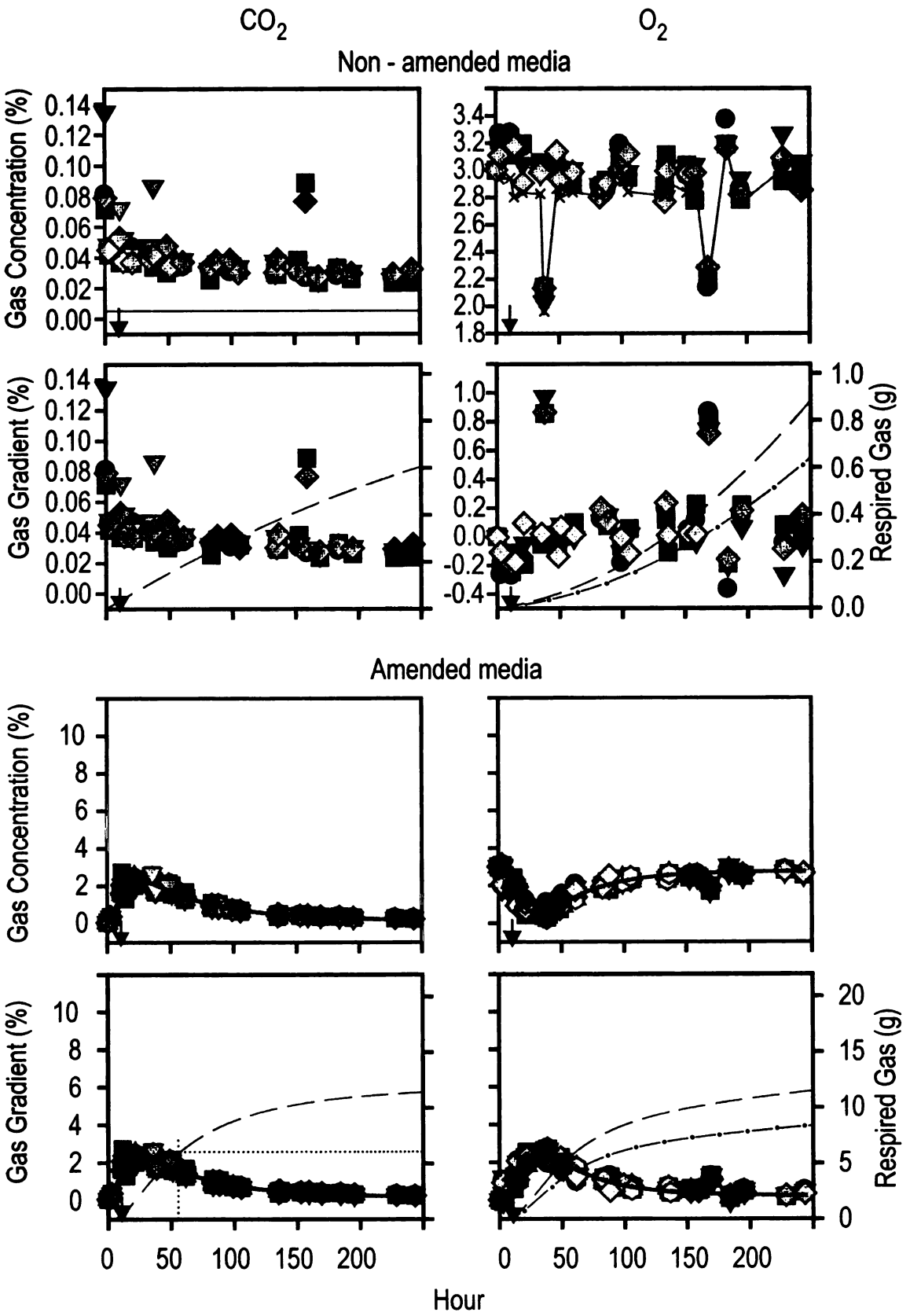
Experiment 2: Low O_2, Low temperature

Specific to this experimental in addition to the general methods described twenty four bioreactors were designated into one of six sets (Set 1 to 6) of four. The gas entering was modified atmospheric gas with 3% oxygen. Set 1 to 5 had amended medium and Set 6 had non-amended medium. The "guide" and treatment reached the target temperature of 25 °C at 10.75 h (Figure 2.4) and the internal media temperature range for the treatment bioreactors range was 23.0 to 25.5 °C for the remainder of the experiment. The single "guide" bioreactor peaked at of 38.9 °C at 34.25 h and dropped below 25 °C at 85 h to the end of the study at 243 h.

The measured difference from the 3% O_2 target post mixing chamber was +/- 0.11% in all but two samples that were attributed to the effect upon one of the two regulators of the change in room temperature (door left open during sampling). Exit O_2 from the bioreactors filled with non-amended media closely tracked that of the mixing chamber with the largest absolute difference of 0.33%. The range of percent CO_2 was between 0.023% to 0.053% for all but eight (0.071% to 0.13%) of the one hundred samples. Based on the equation (#8005 Table Curve 2D v5.01) fitted to the data from the bioreactors with amended medium the lowest O_2 level was 0.54% at 30.62 h and CO_2 levels peaked at 2.26% at 24.36 h and both were maintained at these levels for at least 14 h. As expected there is an inverse relationship in the development between the two gasses (Figure 2.4). The collected data points and fit equation from the O_2 percent measurements were transformed to gradient by subtracting 3% concentration (Figure

2.4). The potential mass of CO_2 respired by the amended media was calculated to be 11.92 grams with half of the maximum CO_2 respired at 56 h (Figure 2.4 Table 2.1).

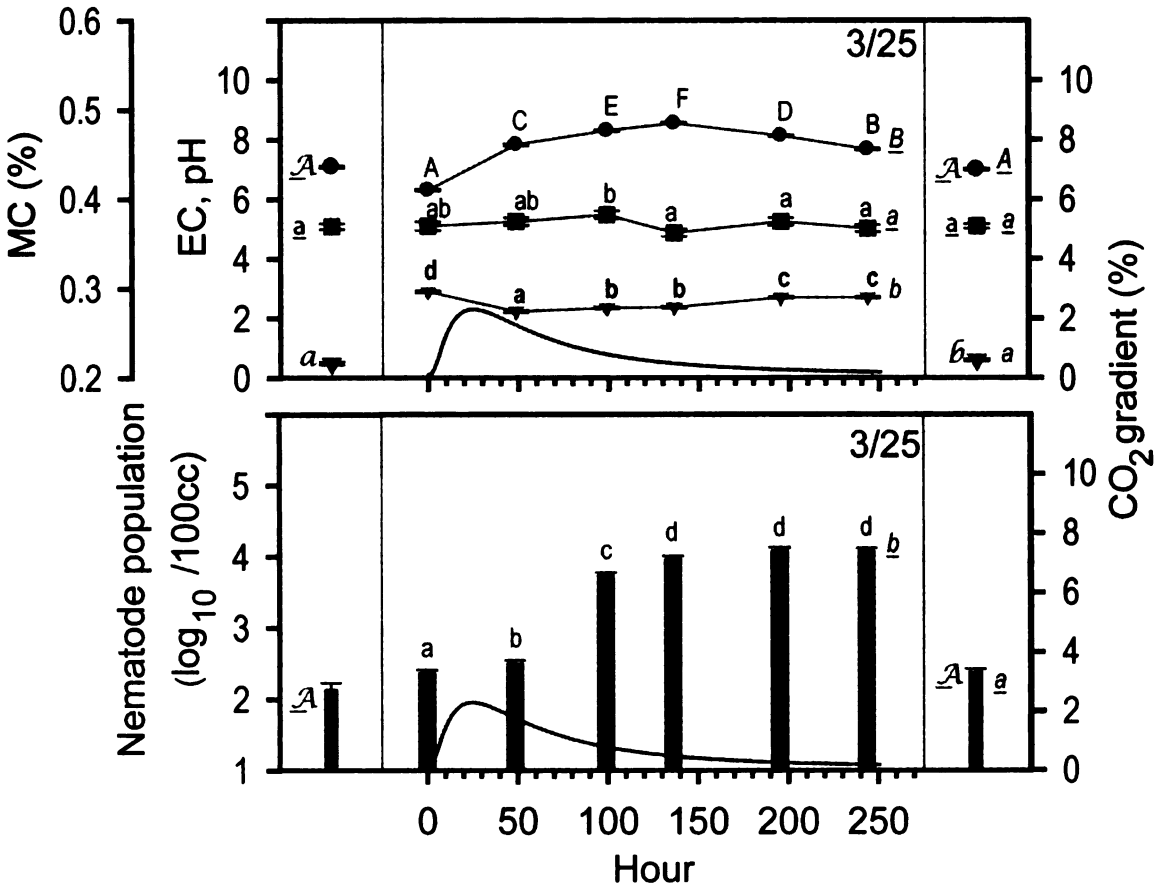
Figure 2.4. Gas concentration (%), gas gradient (%) and respired respiratory gas (g) of non-amended or amended medium under treatment of 3% O_2 influx and 25 °C. Solid fine line is the given atmospheric CO_2%. The solid line interspersed with Xs is the O_2% flowing into media. Arrow indicates hour at which the target internal temperature was reached by self-heating (amended) at which point it is maintained at target value. For bioreactors filled with non-amended media the respired respiratory gas (g) was calculated based upon the slope of a best fit line (not shown) of the data using Excel™. The dashed line with dots is the accumulated consumption of oxygen based on the best fit line. Long dashed line is the accumulated release of carbon dioxide (g) based on either the accumulative sum of the CO_2 or O_2 gas gradient (%) equation. For bioreactors filled with amended media the bold solid line is the fitted equation (#8005 Table Curve 2D v5.01 SYSTAT, Software Inc.) based on sample data. The dashed line with dots is the accumulated consumption of oxygen (g) based on the O_2 gas gradient (%) from the media. Long dashed line is the accumulated release of carbon dioxide (g) based on either the accumulative sum of the CO_2 or O_2 gas gradient (%) equation. The fine doted line indicates the hour and value (g) at which half of the maximum CO_2 respiration was reached.


CO_2
O_2
Non - amended media
Gas Concentration (%)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
Gas Gradient (%)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
Respired Gas (g)
1.0
0.8
0.6
0.4
0.2
0.0
Amended media
Gas Concentration (%)
10
8
6
4
2
0
Gas Gradient (%)
10
8
6
4
2
0
Respired Gas (g)
20
15
10
5
0
0
50
100
150
200
0
50
100
150
200
Hour

The electrical conductivity (EC) of the amended media at the initial mix was 2.90 dS · m^{-1} and followed the trend of decrease at 48.67 h (2.23 dS · m^{-1}) and small gradual increase until end of duration at 243.60 h (2.70 dS · m^{-1}). The initial amended medium had a pH of 6.30 which increased to 8.55 at 136.33 h and then dropped to 7.67 at 243 h. The moisture content of amended medium was held between 36 and 38% and non-amended medium remained at 37% (Figure 2.5).

The nematode population density (log_{10}/100 cm^3) of amended media increased from 2.37 to 2.51, 3.74, and 3.97 over 136.3 h with no further changes to experiment end (243.6 h). There was no change in the non-amended media (Figure 2.5). The actual average nematode population extracted from 100 cm^3 of the medium taken over the 243.6 h is note worthy. The non-amended and amended medium at time zero had the respective values of 146 and 239. On 48.67 h the mixture contained a population of 323 which is a 1.36 fold increase over that of the mixture at time zero. The population increase of 5591 (99.00 h), 9478 (136.3 h), 12283 (195.2 h), and 12470 (243.6 h) is a 23.4, 39.7, 51.4, and 52.2 fold increase over that of the starting mix. At 243.6 h, the non-amended media that underwent treatment within the bioreactor had an average value of 243.

Figure 2.5. EC ($dS \cdot m^{-1}$), moisture content, pH, and nematode population (circle, square, triangle, bar) of non-amended and amended medium under treatment of 3% O_2 influx and 25 °C. Font size and style designate the mean comparisons. Graph segment without designated x axis units is that of the non-amended medium before and after treatment. The solid line represents the CO_2 gas gradient (%).


MC (%)
EC, pH
CO_2 gradient (%)
Nematode population (log_{10} /100cc)
3/25
3/25
Hour
0
50
100
150
200
250

Experiment 3: Low O_2, High temperature; Activated Compost Water Extract

Specific to this experimental in addition to the general methods described nine bioreactors were designated into one of five sets (Set 1to 5) with one for the first four (1a, 2a, 3a, and 4a for harvest at time 0, 27.25, 37.75, and 95.42 h) and five for the final harvest at 118.7 h (5a to e). All had the same amended medium and the incoming gas of 3% O_2 (+/- 0.13). The "guide" bioreactor temperature peaked at 38.7 °C at 42.5 h (Figure 2.6) and for the remainder of the experiment the treatment bioreactors were maintained at the predetermined target of 40 °C.

Based on the fitted equation (#8005 Table Curve 2D v5.01) the lowest exit O_2 level was 0.24% at 42.5 h and CO_2 peaked at 2.8% at 36.5 h. Based on the samples taken at individual time points percent O_2 was below 0.5% for 28 h from hour 29 to 57 and CO_2 was maintained above 2.5% for 23 h from hour 25.5 to 48.5. The percent gradient for both CO_2 and O_2 are presented in Figure 2.6. The potential mass of CO_2 respired by the amended media was calculated to be 17.12 grams. The hour at which 1/2 maximum CO_2 respired was at 65.5 h (Figure 2.6 Table 2.1).

Table 2.1. Table Curve 2D v5.01 (SYSTAT, Software Inc.) values for constants of equation 8062 a) apex height, and b) apex hour; equation 8005 are a) y-intercept, b) apex height, and c) apex hour. Values within parenthesis (_) are to correct the respective equations for the background CO_2 of non-amended medium or to obtain a better fit for the data.

Experiment 1: 21%O_2/25 °C
Equation # 8062 (Table curve 2D v 5.01)

	a	*b*	*c*	*d*	r^2	Max grams	Hour 1/2 Max
CO_2	5.028	8.591	0.742 (0.8612)	50.331	0.958	(15.50)	(35.35)
O_2	7.117	8.020	0.617	55.118	0.900		

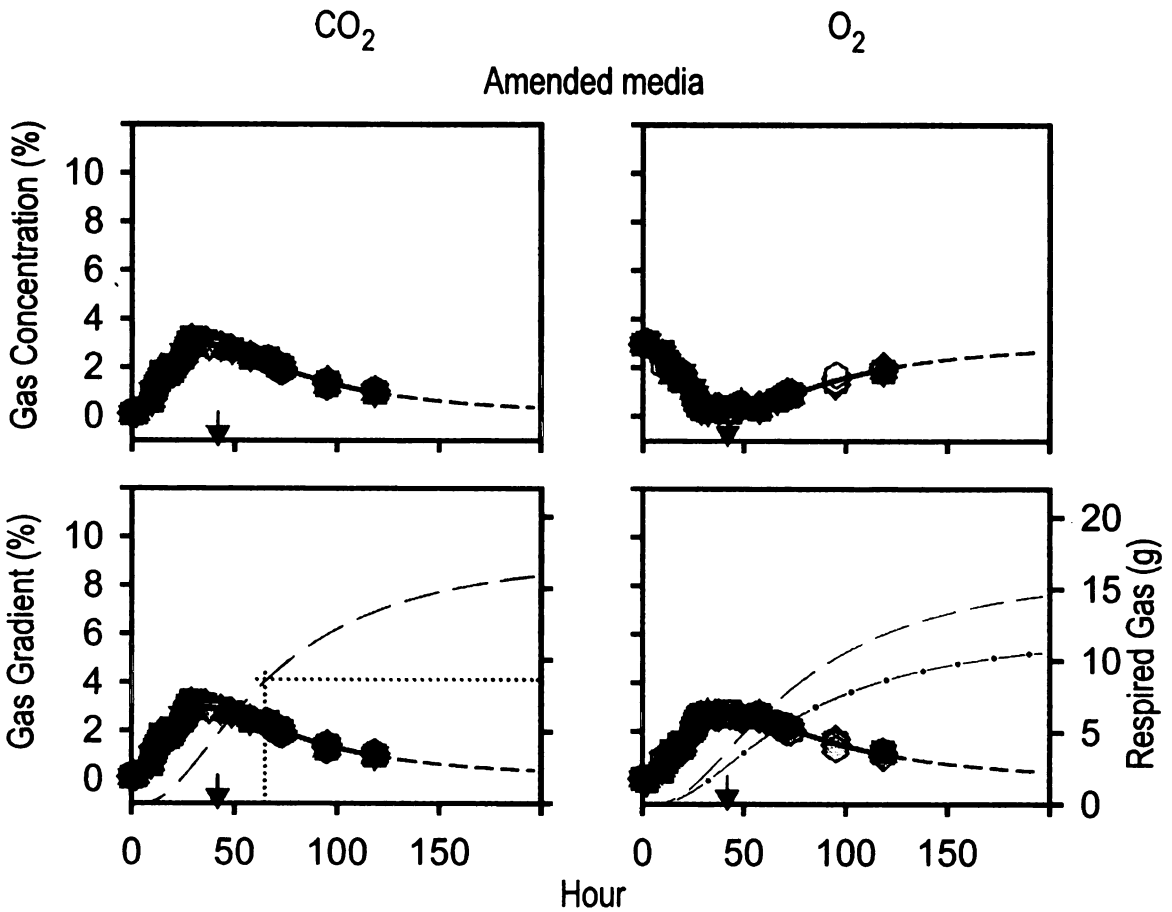
Experiment 2: 3%O_2/25 °C
Equation # 8005 (Table curve 2D v 5.01)

	a	*b*	*c*	*d*	r^2	Max grams	Hour 1/2 Max
CO_2	0.076 (0.0)	2.187	24.863	0.905	0.917	(11.92)	(56.10)
O_2	0.209	2.253	30.927	0.698	0.930		

Experiment 3: 3%O_2/40 °C
Equation # 8005 (Table curve 2D v 5.01)

	a	*b*	*c*	*d*	r^2	Max grams	Hour 1/2 Max
CO_2	0.106 (0.0)	2.833	36.366	0.767	0.977	(17.12)	(65.45)
O_2	0.030	2.732	42.137	0.739	0.977		

Figure 2.6. Gas concentration (%), gas gradient (%) and respired respiratory gas (g) of amended medium under treatment of 3% O_2 influx and 40 °C. Arrow indicates hour at which the target internal temperature was reached by self-heating (amended) at which point it is maintained at target value. The bold solid and extended dashed line is the fitted equation (#8005 Table Curve 2D v5.01 SYSTAT, Software Inc.) based on sample data. The dashed line with dots is the accumulated consumption of oxygen (g) based on the O_2 gas gradient (%) equation from the media. Long dashed line is the accumulated release of carbon dioxide (g) based on either the accumulative sum of the CO_2 or O_2 gas gradient (%) equation. The fine doted line indicates the hour and value (g) at which half of the maximum CO_2 respiration was reached.


CO_2
O_2
Amended media
Gas Concentration (%)
Gas Gradient (%)
Respired Gas (g)
Hour
0
2
4
6
8
10
15
20
50
100
150

At time zero the EC and pH of the non-amended medium changed with the addition of amendment from 0.5 to 3.1 $dS \cdot m^{-1}$ and 7.2 to 6.7 respectively. The EC of amended medium decreased to 2.3 $dS \cdot m^{-1}$ at 37.7 h and followed by a gradual increase to 2.7 $dS \cdot m^{-1}$ at 118.7 h. The pH amended medium increased from 6.7 to 8.9 from time zero to 118.7 h. Moisture content of amended medium was maintained between 38.7% and 40.9% and that of non-amended medium was 36.2% (Figure 2.6).

The evaluation of the total bacterial population dynamics of uniformly mixed amended medium was done for time zero, each subsequent time interval, and one of the five bioreactors at the last sampling interval. The samples were prepared by placing 0.1 L medium and 1.0 L of RO purified water in a 2.5 L container and shaken for twenty minutes on a horizontal shaker with a 4 cm stroke at 80 rpm. After removal it was placed vertically on a table and allowed to settle for five minutes before pouring off 0.8 L from which a single milliliter was placed in a sterilized 0.1 L corning ™ tube and mixed to make 1:10 3% formaldehyde solution. The prepared solution was stored at 5 °C for no more than two weeks. The $\log_{10}$ count (n=20) of the 1:10 solution at time zero was 7.08 followed by the respective bioreactors with 7.10 (27.25 h), 7.25 (37.75 h), 7.65 (73.33 h), 7.59 (95.41 h), and 7.62 (118.7 h). As a trend there is little increase through to the third sample and the population increase thereafter remains stable over the remaining two samples (Figure 2.7).

Figure 2.7. EC ($dS \cdot m^{-1}$), moisture content, pH, and bacterial population (circle, square, triangle, bar) of amended medium under treatment of 3% O_2 influx and 40 °C. Graph segment without designated x axis units is that of the non-amended medium before treatment. The solid and dashed lines respectively represent the calculated and projected gradient (%) of CO_2.

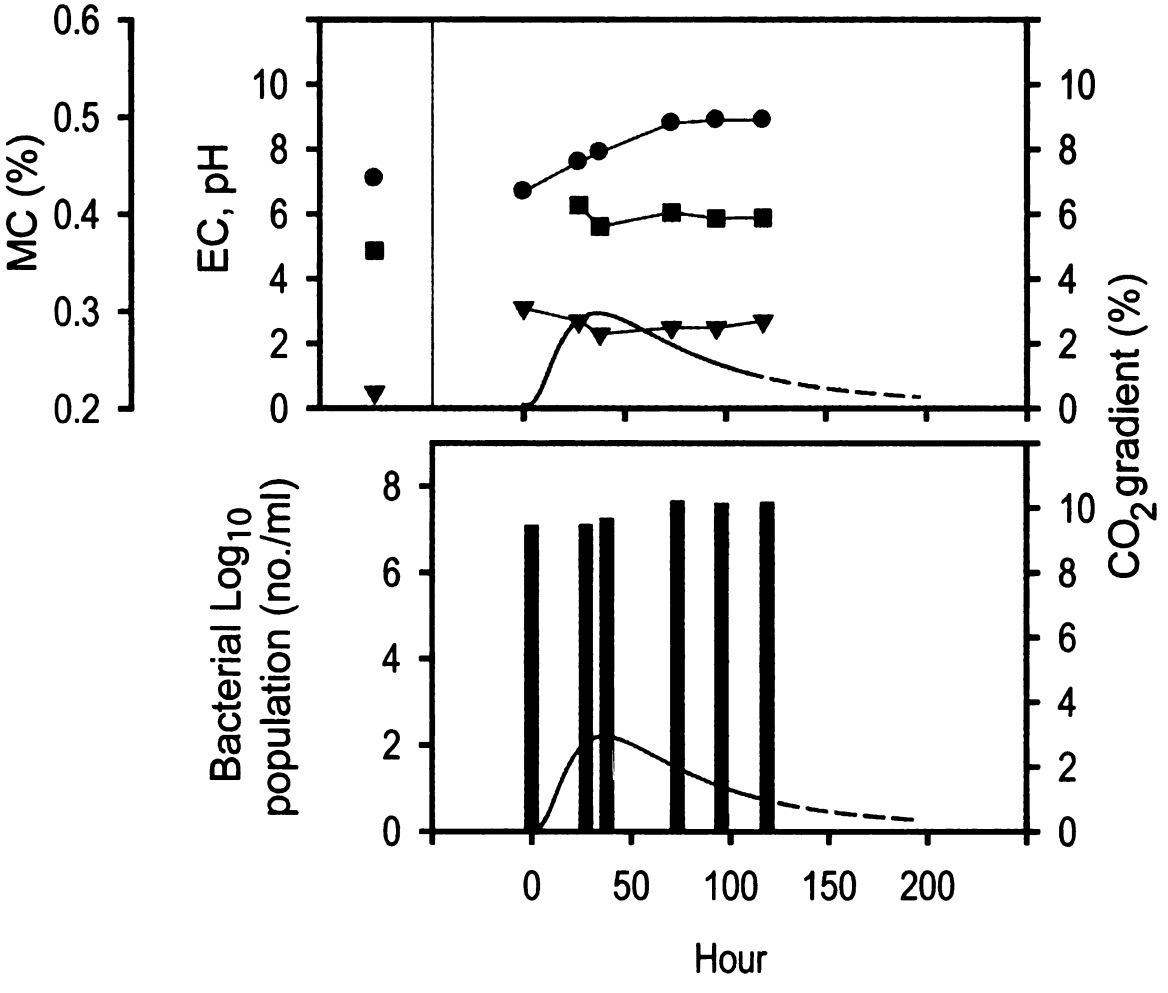

MC (%)
0.6
0.5
0.4
0.3
0.2
EC, pH
10
8
6
4
2
0
CO_2 gradient (%)
Bacterial Log_{10} population (no./ml)
0
50
100
150
200
Hour

Figure 2.7. EC ($dS \cdot m^{-1}$), moisture content, pH, and bacterial population (circle, square, triangle, bar) of amended medium under treatment of 3% O_2 influx and 40 °C. Graph segment without designated x axis units is that of the non-amended medium before treatment. The solid and dashed lines respectively represent the calculated and projected gradient (%) of CO_2.

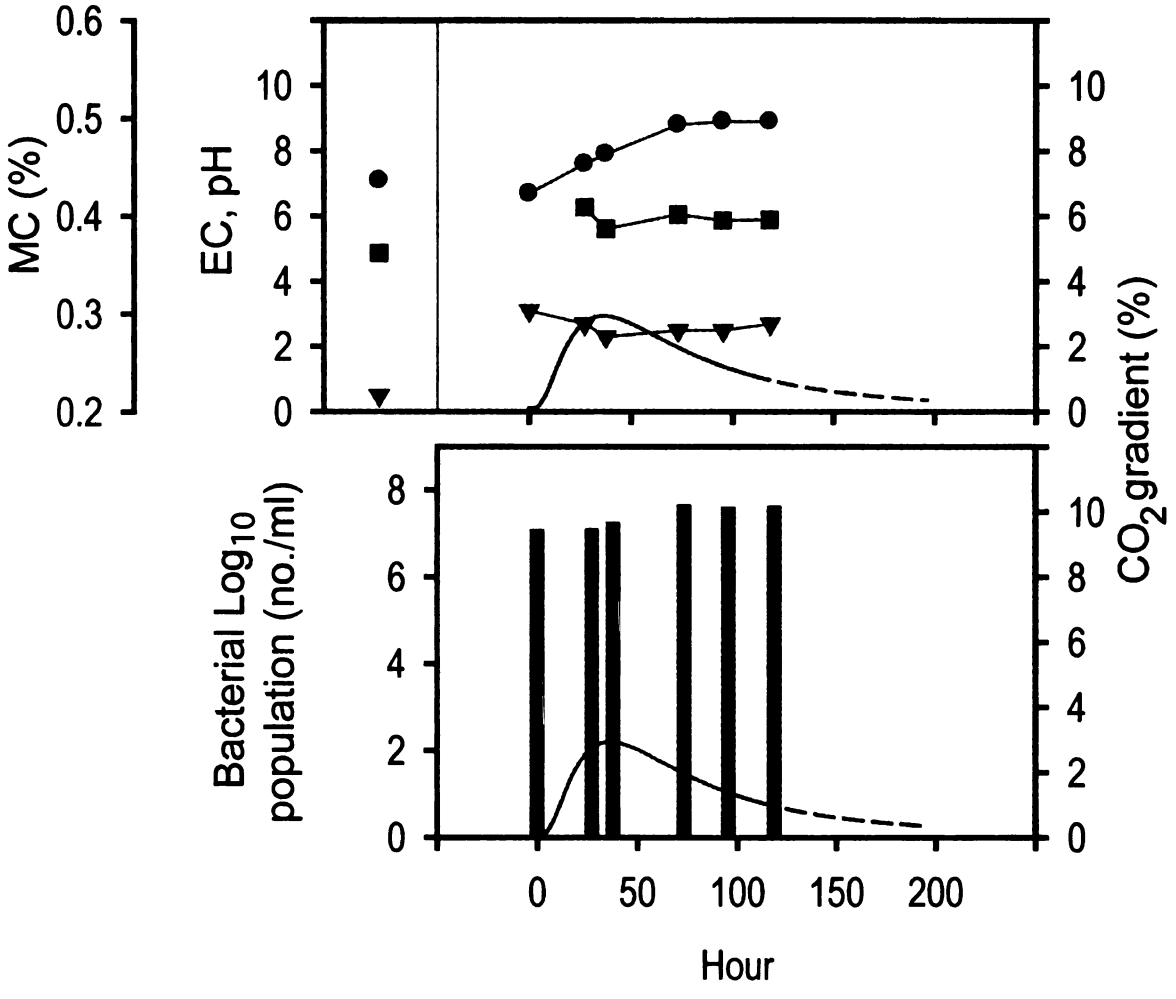
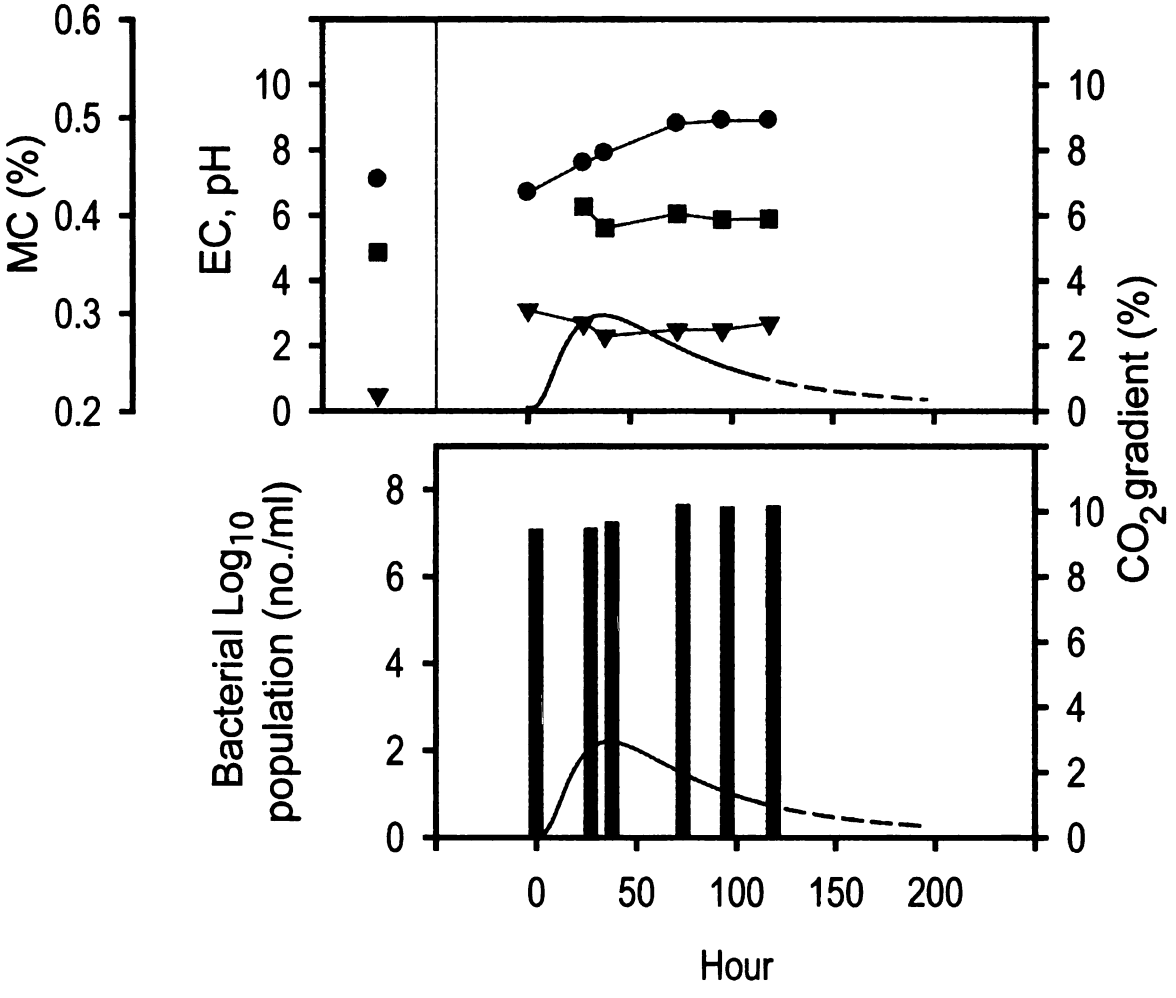

MC (%)
0.6
0.5
0.4
0.3
0.2
EC, pH
10
8
6
4
2
0
CO_2 gradient (%)
Bacterial Log_{10} population (no./ml)
0
50
100
150
200
Hour

Figure 2.7. EC ($dS \cdot m^{-1}$), moisture content, pH, and bacterial population (circle, square, triangle, bar) of amended medium under treatment of 3% O_2 influx and 40 °C. Graph segment without designated x axis units is that of the non-amended medium before treatment. The solid and dashed lines respectively represent the calculated and projected gradient (%) of CO_2.


MC (%)
0.6
0.5
0.4
0.3
0.2
EC, pH
10
8
6
4
2
0
CO_2 gradient (%)
Bacterial Log_{10} population (no./ml)
0
50
100
150
200
Hour

Discussion

The goal was to test a bioreactor capable of controlling the variables of gas flow rate, maximum O_2%, relative humidity, and maximum temperature upon a substrate of non-amended and amended compost medium. The gas flow through the medium was regulated for both maximum O_2% for consumption and rate ensuring volatile byproduct stagnation did not occur. A battery of bioreactors provided the data to confirm their reliability and desired level of accuracy. The processed data was to provide insight to the effects of the controllable variables upon non-amended and amended medium.

The non-amended medium is in a relative state of equilibrium with a predominantly dormant microbial population. With the addition of an amendment to the medium there is increased microbial activity. Both of these media were evaluated for microbial activity by measuring the O_2% and CO_2% under different oxygen and thermal conditions, and found to be within the anticipated norms of processes of compost(ing) materials.

As anticipated the O_2 and CO_2 respiration rate of a non-amended medium was stable over the duration of the experiments. Unanticipated from this medium under the treatments of 3% and 21% O_2 at 25 °C are the calculated total CO_2 respiration of 0.6 g and 1.2 g respectively. Using the same gas flow rate provides insight to conditions where oxygen is, but porosity is not limiting. It was assumed dormant microbial population respiration would not be influenced by O_2 at 3% due to the lack of readily available energy. If these findings were to be substantiated, the foundation for study into

the selection by O_2 levels occurring at the population level of strict and facultative aerobic microbes within mature compost.

The CO_2 projected maximum respiration and hour of 1/2 maximum for the three treatments are 15.50 g and 35.4 h (21/25), 11.92 g and 56.0 h (3/25), 17.12 g and 65.5 h (3/40). The projected total grams of CO_2 released under the low temperature treatment (25 °C) is greater under the ambient (>13.5%) than the low O_2 treatment (<3%). The greater rate of respiration between the two treatments is illustrated by the hour at which 1/2 maximum occurred which was 25.7 h earlier under the ambient O_2 treatment. The hour at which the target temperature (25 °C) was reached from the initial (18-19 °C) by the low and high O_2 treatments is a half an hour (10.75 vs. 10.25 h) during which the O_2 decrease was 1.5 and 7.5% respectively. The biological processes within amended media under treatments of 3% O_2 at 25 or 40 °C reduced the O_2 gradually to 0.54% (30.62 h) and 0.24% (42.5 h) respectively. The O_2 values and the hour are relatively close despite one being maintained at 25 °C as the other continued to increase to 40 °C for an additional 31.25 h. The treatment of low O_2 elevated target temperature (3/40) fell short by 1 °C and is the maximum heat output under the specific 3% O_2 configuration.

The change in pH of the amended medium under 3% O_2 at both temperature treatments gradually changed and peaked after 125 h in contrast to that of 21% which peaked before 48.75 h. Changes of the pH within all three experiments of amended media are in the normal range of composting.

The moisture content of non-amended and amended medium was maintained for the experiment duration within 2% of the selected percentage. The differing biological activity (respiratory release of H_2O as a byproduct) within the two media did not lead to either a reduction or increase of moisture content, indicating that the self regulative relative humidity system functioned in spite of the mechanically regulated gas flow through the system. This is not typical for composting. For composting the initial moisture content is calculated to anticipate the loss of water in vapor form, occurring during the biologically active phase, and not become a limiting factor during the remaining phases.

Not substantially affected by O_2 settings or a fluctuation of extended near hypoxic conditions at 25 °C, is the exponential growth of bacterial feeding nematodes in amended media. Both (3% and 21%) underwent an initial lag phase up to 50 h, peaking with a slightly lower value, after 130 h and 190 h respectively. This predatory population is able to react quickly to an indirect influx of energy within the system. Based on the total bacterial count (without replicates) of amended media (O_2 3% 40 °C) an onset of the exponential growth occurred well beyond what would be considered a normal lag phase and a short growth phase. This could be accounted for by the coinciding nematode population growth based on the other two experiments or in addition to the nematodes, the heterogeneous microflora may undergo an environmental selection for this particular treatment whereby the biomass of one bacterial population is sequestered by that favored under the changing conditions.

Further studies are recommended to include additionally the treatment 21% O_2 and 40 °C in the production and evaluation of activated compost water extracts. Under low oxygen conditions of the just described experiment a distinctive shift in the odor of the exit gas occurred. Based on this observation, it is likely that there are shifts in the volatile compounds given off by the microbial populations of the medium which could be identified and quantified. As a result of a multitude of environmental niches the composted materials have under gone, the mature compost as the foundation for a medium provides a heterotrophic population adapted to making population shifts over time. A method capable in differentiating the bacterial population shifts within and between the treatments is recommended.

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Chapter III

Incubation of Mature Compost and Alfalfa Amendment for Production of Activated Compost Water Extract: Final Summary Experiment with Incubation at High or Low Temperature and High or Low Oxygen

Abstract

Mature compost (substrate) and a certified organic alfalfa based fertilizer (amendment) were mixed and placed under four conditions, defined by one of two target O_2 and temperature settings maintained within bioreactors. Sampling interval was based on O_2 consumption and included pH, electrical conductivity (EC), O_2 and CO_2 respiration, total bacterial count, fungal biomass, stability of moisture content and bacterial populations. The bacterial populations was characterized using T-RFLP and gas volatiles using gas chromatograph, mass spectrometry (GC/MS) to further specify differentiation and change as a result of treatment. The protocol and findings provide a foundation for the manipulation of mature compost for the purpose of preparation of a water extract for the study of its influence upon the interaction of plant pests/pathogens and host. The method provides an alternative to the production of aerated compost tea.

Introduction

The application of compost changes properties of soil by increasing diverse local microbial population, porosity, water retention, nutrient caring capacity, long term nutrient availability, crumb structure, internal stability of readily available nutrients, and physical structure (Stoffella and Kahn, 2001). It has been experimentally demonstrated that the application of compost to soil or as soilless potting media, contributes to impeding the development of disease by pathogen and host (Hoitink, 1996; Litterrick, 2004). The mechanisms attributed to the mode of action or combination there of are poorly understood but are proposed to be direct nutrient competition, antibiosis between

one or more microorganisms, hyperparasitism, induced protection from within the plant (Hoitink, 1996; Litterrick, 2004).

In an effort to extend some of the benefits that compost provides to plant health an aqueous solution of compost is made and used directly on the foliage in an effort to impact a foliar disease. In broad terms the product is made using compost as the microbial inoculant and water as the medium wherein the biological processes take place before use. A wide range of protocols exist calling for various forms of microbial energy sources (sugars), minerals and the desired O_2 saturation throughout the production phase. Currently the use of such products outreaches the scientific testing and evaluation done on the efficacy of various protocols. One of the leading protocols used here in the USA, aerated brewed compost tea, calls for the use of quality compost, various recommended amendments, O_2 levels in solution maintained near saturation, and use of a proven bioreactor (Ingham, 2003).

An alternative is proposed in which key variables can be altered and controlled in a reproducible fashion. It calls for the use of the compost both as the inoculum and the medium within a solid state bioreactor. An energy source (amendment) is added to the compost so that active microbial population dynamics and a selective process takes place depending on the reproducible environmental conditions within the bioreactor. As Charles Darwin (1859) eloquently stated in The origin of species by means of nature selection, or, The preservation of favored races in the struggle for life: "In the struggle for survival, the fittest win out at the expense of their rivals because they succeed in adapting themselves best to their environment." and "I have called this principle, by which each slight variation, if useful, is preserved, by the term Natural Selection."

Without directly isolating a specific microbe, a locally cultivated heterotrophic microbial population under goes a process of natural selection under specific and know conditions. The treated amended compost is then placed in water for a short agitation period to extract the microbial population and byproducts for foliar application to plants. With organic certification, the resulting extract is subject to fewer food safety concerns then brewed teas. The long term goal is to identify a reproducible protocol for compost based product that is to be extracted and suspended in water which is effective in impeding the plant host and pathogen interaction either on specific or general targets.

In previous studies (Jost, Biernbaum unpublished), protocols were developed to a) incorporate the amendment to the compost substrate, b) identify an appropriate amendment rate, c) determine a method of maintaining air flow and gas concentration in the chamber, d) determine protocols for measuring gas concentrations in the incubation chamber, e) determine a method to maintain moisture content in the substrate, f) determine a method of regulating temperatures after self heating in the chamber, g) characterize post incubation electrical conductivity (EC), pH, and microbial populations in the non-amended and amended substrate mixture, h) identify a mathematical equation for the gas concentration that best fits the data, and i) characterize percent, gradient and respiration for both O_2 and CO_2; the hour of half maximum and the maximum CO_2 released, and j) determined a method for a water extraction of the treated compost.

Additional protocols are reported here to a) identify volatile gaseous compounds from the incubation chamber and, b) PCR characterization of the bacterial microbial community from two of the simultaneously conducted four treatment conditions.

Cultural independent methods for characterizing and identifying microbial populations during the composting process has been done using biochemical or molecular techniques such as phospholipid fatty acid analysis (PFLA) (Klamer and Baath, 1998), or polymerase chain reaction (PCR) based techniques (Marsh, 2005; Tebbe, 2002). LaMontagne, et al. (2002) has evaluated various extraction and purification protocols for using terminal restriction fragment length polymorphisms (TRFLP) for the genetic characterization of compost.

Characterization and diversity of microbial communities under hot composting conditions (maximum temperature of 80 °C at day 10; 65 °C at day 18) of shredded maize plants, wood chips, and straw-bedded horse manure (6.4 m^{-3}), found bacterial diversity increased over the 18 day study. The actinomycetes population succession had little increase in diversity through day 18 at which point a dramatic increase occurred (Peters, 2000).

Roberts (2002) conducted an in-vessel rotating drum composting study under aerobic conditions with imposed temperature treatments (25 or 55 °C) with various time intervals of either or both settings for 30 days for 11 experiments. The bacterial population richness was high for the first 5 days followed by a decline through the thermophilic phase to day 12. The diversity increased and was maintained from day 30-90.

At a large scale facility Michel (2002) reported bacterial communities changed over the 136 days of composting of mixture of leaves, grass, and brush. At time 0 the population primarily consisted of gram-negative α, β, and γ Proteobacteria within the community which underwent a substantial shift and by day 8, 29, and 64 the community

contained many Gram-positive *Bacillus-Clostridium* members and also members of *Clostridium difficile* and Actinobacteria. The greatest diversity was found on days 64 and 136 thereafter.

The objective of this study was to manage temperature, O_2 , and input relative humidity and measure medium temperature, EC, pH, MC, O_2, and CO_2 and either total biomass or number of individuals of bacterial, or fungal of the non-amended and amended compost over time. Shifts of bacterial communities and gaseous bi-products were identified by genetic and molecular profiling.

Materials and Methods

The following materials and methods are previously described (Chapter 2): location, constructed bioreactors, compost (non-amended medium), compost mix (amended medium) and placement, target temperature and control/guidance, relative moisture content of amended medium, gas O_2% levels, gas flow rate, real time monitoring in situ sampling, gas monitoring and data processing, analysis of non-amended and amended medium electrical conductivity (EC), pH, and moisture content. Nematode population density was not enumerated. Also described was the preparation of the activated compost water extract and bacterial enumeration of the extract.

Different from the previously described protocol is the simultaneously conducted treatments of temperature and O_2, analysis of exit gas volatiles, activated compost water extract solution pH, EC and biological analysis. A 0.5 L non-amended or amended medium (compost) water extract sample was shipped overnight in a 1 L plastic bottle for evaluation of the total and active fungal population (Soil Foodweb New York, Inc.,

555-7 Hallock Avenue, Port Jefferson Station, NY 11776, USA) (Ingham, 1982; Ingham, 1986) Four aliquots were taken from the non-amended and amended medium at time zero, and all subsequent intervals for amended medium from the bioreactors and placed in sterilized 0.5 L corning ™ tube that was stored in a -80 °C freezer for genetic and bacterial population characterization using T-RFLP (Marsh, 2005).

For each of the post harvest non-molecular tests preformed on the medium, one way analysis of variance with four phase intervals (P0, P1, P2, and P3) was done with t-tests using PROC GLM in SAS with least square mean statement for phase comparisons. One phase (P2) within each treatment was replicated and variance for the other phases is assumed to be representative. The assumption is made based upon various phases from previous experiments.

The amended medium temperature was maintained at either 25 or 40 °C after self heating to target temperature. The treatments and designation (O_2% / Temperature °C) are as follows: 3/25, 21/25, 21/40, and 3/40. Five bioreactors in each of the four treatments were used to take post treatment samples of the medium. A temperature "guide" bioreactor for each respective O_2 regime (3% or 21%) was used to establish the rate of temperature increase. The maximum medium temperature attained for the two bioreactors was 39 °C (3%) and 69 °C (21%). Based on the physiological consumption of O_2 consumption, a single sample interval of the non-amended medium and the four sample intervals of amended medium were taken and designated as Phase 0, 1, 2, and 3 (P0, P1, P2, and P3). The designation, P0 is the prepared non-amended and amended medium and no change (0 h) in the O_2 consumption has occurred. At P1 the O_2%

reached the lowest measurement within the specific treatment. At P2 and P3 the targeted gas levels returned from P1 to one-third and two-third of the initial O_2% (3 or 21%) respectively. At P0 from non-amended and amended medium 3 samples were taken. From amended medium at interval P1, P2, and P3 the number of samples taken were 1, 3, and 1 respectively.

Sampling of volatile compounds from one bioreactor for each treatment (3/25, 3/40, 21/25, and 21/40) was accomplished using solid phase microextraction (SPME, Supelco Co., Bellefonte, PA) as described by Song et al. (1997). The SPME fiber, coated with 65 μm-thick polydimethylsiloxane/divinylbenzene, was inserted into the exit line of the respirometer and volatiles were absorbed for 3 min. The exposed fibers were transferred to a gas chromatograph (GC) (HP-6890, Hewlett Packard Co., Wilmington, DE) injection port (230 °C) and desorbed for 2 min. Desorbed volatiles were trapped on-column using a liquid nitrogen cryofocusing trap. Separation of volatiles was by capillary column (SupelcoWax-10, Supelco, Bellefonte, PA, 30 m x 0.25 mm i.d., 0.25 μm-thick stationary phase). The initial temperature of the GC was 40 °C, increasing immediately at a rate of 40 °C/min to 240 °C and held for 1 min; the flow rate of the helium carrier gas was 1 $mL \cdot min^{-1}$ and the GC was operated in splitless mode. Detection was by time-of-flight mass spectrometry (Pegasus II, LECO Corp., St. Joseph, MI) (GC/MS) according to the method of Song et al. (1997) . Compound identification was by comparison of the mass spectrum with those of authenticated reference standards and spectra in the National Institute for Standard and Technology (NIST) Mass Spectra Library (Search Version 1.5). Volatile quantities are reported as the response (total ion count, TIC) of the mass spectrometer.

Gas samples were taken and analyzed for the ionic content from a single bioreactor from each of the four treatments (3/25, 3/40, 21/25, and 21/40); one of each O_2 treatments at hour one. Samples were taken intermittently every 6 to 24 h. Based on the selected ion a tentative identification was made of a compound.

The bacterial population from two treatments (3/40 and 21/40) was characterized using terminal restriction fragment length polymorphism (T-RFLP) as described by Marsh (2005). The treatment 3/40 had all four sample phases taken (P0, P1, P2, and P3), whereas three were taken (P0, P2, and P3) from treatment 21/40. Community DNA was extracted using MoBio Soil DNA Isolation kits following the protocol recommended by the vendor. DNA concentration was estimated spectrophotometrically (A_{260nm}). Amplification of the 16S rRNA genes was achieved using general bacterial domain primers (27F & 1387R) (Marchesi, 1998)) with the following thermocycling conditions; 1 cycle at 95 °C for 5 minutes followed by 30 cycles at 95 °C-50 sec, 58 °C-50 sec, 72 °C-1.5 min and ending with a 7 min incubation at 72 °C. PCR products were purified with a Qiagen PCR Clean-up kit and quantified spectrophotometrically (A_{260nm}). Approximately 300 ng of PCR product was used in 20 µl restriction digestions using Hha I or Msp I according to the manufacturer's recommendations (NE Biolabs). Samples were then sent to the MSU RTSF for fragment separation and sizing on an ABI 3100 capillary electrophoresis system.

Data received by the lab (Hha I) was processed to designate fluorescent peaks (arbitrary units) to fragment lengths (bases) using Gene Scan 3.1 (ABI PRISM) with a selective criteria of peak height minimum of 50 and a distance no greater than 0.5

fragment length. In the case where one of the technical replicates had an identified peak greater than 50 and the other replicate peak did not meet the height requirement it was given a designated value of 49. Within a fragment length group where it is detected in at least one of the phases (P0, P1, P2, or P3 in both reps) and not in another, the missing detection is again assigned a value of 49. To identify the larger contributors of the bacterial population from each treatment (3/40 or 21/40) a selection from the identified fragment lengths (bases) was made based on whether one or more of the technical average fluorescent fragment length height was 2.5 times greater (125) than the initial selection criteria (50). From this selected group a descriptive analysis of the individual base pairs was made based upon their dynamics through the sampled phases (P0, P1, P2, or P3) of the treatments of maximum oxygen of 3 or 21% at 40 °C. When one or more of the technical replicates average height is 49, it was designated "transient", as non-detection and detection over the various samples is characteristic. A fragment length was identified as "persistent" when the average technical replicate base pair height over all the phases was greater than 50. A further designation within "persistent" was made, when the base pair height difference between the lowest and highest was below or above a two fold increase, as "persistent-stable" and "persistent-non-stable" respectively. The inter and intra r^2 value between the technical replicates base pair height was done and the slope equation calculated.

The identified fragment lengths (bases) (restriction digestions Hha I or Msp I) from both treatments were designated into an evolutionary tree based presence/absence of the base pair unweighted pair group method with arithmetic mean (UPGMA) using

the software phylogenetic analysis using parsimony (PAUP 4.0 Beta, David L. Swofford, Florida State University).

Results

The control of temperature with one of the two O_2 flow rates at the designated target of 25 or 40 °C was implemented at hours 15 (3/25), 38 (3/40), 10 (21/25), and 14.5 (21/40) (Figure 3.1). The slower rate of temperature increase was under the lower O_2 treatments. The respective absolute hours of the time difference of the increase from 25 to 40 °C was 23 (3% O_2) and 4.5 (21% O_2) h.

With regulated flow of low O_2 (3%) the O_2 levels were reduced to 0.53% (25 °C) and 0.29% (40 °C) based on the fitted equation (#8005, Table Curve 2D v5.01). The reduced O_2 level (<0.5%) continued for 15 and 49 hours in the respective treatments as an average value taken from the raw data from each sample interval. With regulated flow of high O_2 (21%) the O_2% reduction was to 16.6% (25 °C) and 11.1% (40 °C) as determined by the fitted equation (#8062, Table Curve 2D v5.01). In contrast to the low O_2 the greatest levels of O_2 reduction were not sustained for any length of time. The r^2 and constants are provided in Table 3.1 for the two equations selected from the software library.

Under the continuous inflow of low percentage O_2 (3%) treatments (25 and 40 °C) the CO_2 concentration based on the equation respectively peaked at 2.4% and 2.8% at hours 33 and 35. Based on the average collected data of each sample these levels

were maintained greater than 2.5% for 13 and 28 hours respectively followed by a prolonged decline. Under the treatments with high O_2 concentration (21%) both underwent a sharp increase in CO_2 concentration over a short period of time. As determined by the fitted equation the 25 and 40 °C treatments peaked at hour 11 and 14 with a CO_2 concentration of 4.5 and 9.1%. The peak values were not sustained but followed a sweeping decline. There is graphical similarity of the fitted line within the respective influx of both the low and high O_2 percentage (Fig. 4.1).

The calculated maximum grams of CO_2 respired and the hour at which half maximum occurred within the respective temperature regimes (25 or 40 °C) is greater in both treatments with 3% O_2. At 25 °C the respective release and hour is 17.7 g (3%) and 14.2 g (21%), at 74.0 and 39.8 h. At 40 °C the respective values are 25.0 g (3%) and 18.1 g (21%), at 88.4 and 26.7 h. Within the respective O_2 regimes (3 or 21%) the calculated maximum grams of CO_2 released is greater under the higher target temperature of 40 °C. Under the lower O_2 (<3%) treatment the later hour at which half maximum was reached corresponds to the greater release CO_2. In contrast to the previous treatment the correlation between the later hour of half maximum and the greater total sum CO_2 did not occur under the ambient O_2 (21%) treatment.

Table 3.1. Table Curve 2D v5.01 (SYSTAT, Software Inc.) values for constants of equation 8005 are a) y-intercept, b) apex height, and c) apex hour; equation 8062 a) apex height, and b) apex hour. Values within parenthesis (_) are to correct the respective equations for the background CO_2 of the mature compost or to obtain a better fit for the data.

Treatment: 3% O_2 / 25 °C
Equation # 8005 (Table curve 2D v 5.01)

	a	*b*	*c*	*d*	r^2	Max grams	Hour 1/2 Max
CO_2	0.076	2.451	32.730	0.909	0.851		
	(0.0)					(17.72)	(74.00)
O_2	0.124	2.351	38.458	0.779	0.874		

Treatment: 3% O_2 / 40 °C
Equation # 8005 (Table curve 2D v 5.01)

	a	*b*	*c*	*d*	r^2	Max grams	Hour 1/2 Max
CO_2	0.051	2.882	35.081	0.968	0.931		
	(0.0)					(25.05)	(88.40)
O_2	0.000	2.708	42.267	0.953	0.949		

Treatment: 21% O_2 / 25 °C
Equation # 8062 (Table curve 2D v 5.01)

	a	*b*	*c*	*d*	r^2	Max grams	Hour 1/2 Max
CO_2	4.509	11.352	0.563	65.581	0.909	11.78	34.75
			(0.680)			(14.23)	(39.75)
O_2	4.641	13.041	0.961	57.598	0.926		

Treatment: 21% O_2 / 40 °C
Equation # 8062 (Table curve 2D v 5.01)

	a	*b*	*c*	*d*	r^2	Max grams	Hour 1/2 Max
CO_2	9.134	14.411	1.498	14.988	0.968	16.97	25.90
			(1.600)			(18.13)	(26.70)
O_2	9.900	15.673	1.876	13.720	0.967		

Figure 3.1. Gas concentration (%) of amended medium under four different treatments (percent O_2/ temperature °C). Arrow indicates hour at which the target internal temperature was reached by self-heating at which point it is maintained at target value. The bold solid line and extended dashed line is the fitted equation (#8005 or # 8062 Table Curve 2D v5.01 SYSTAT, Software Inc.) based on sample data.

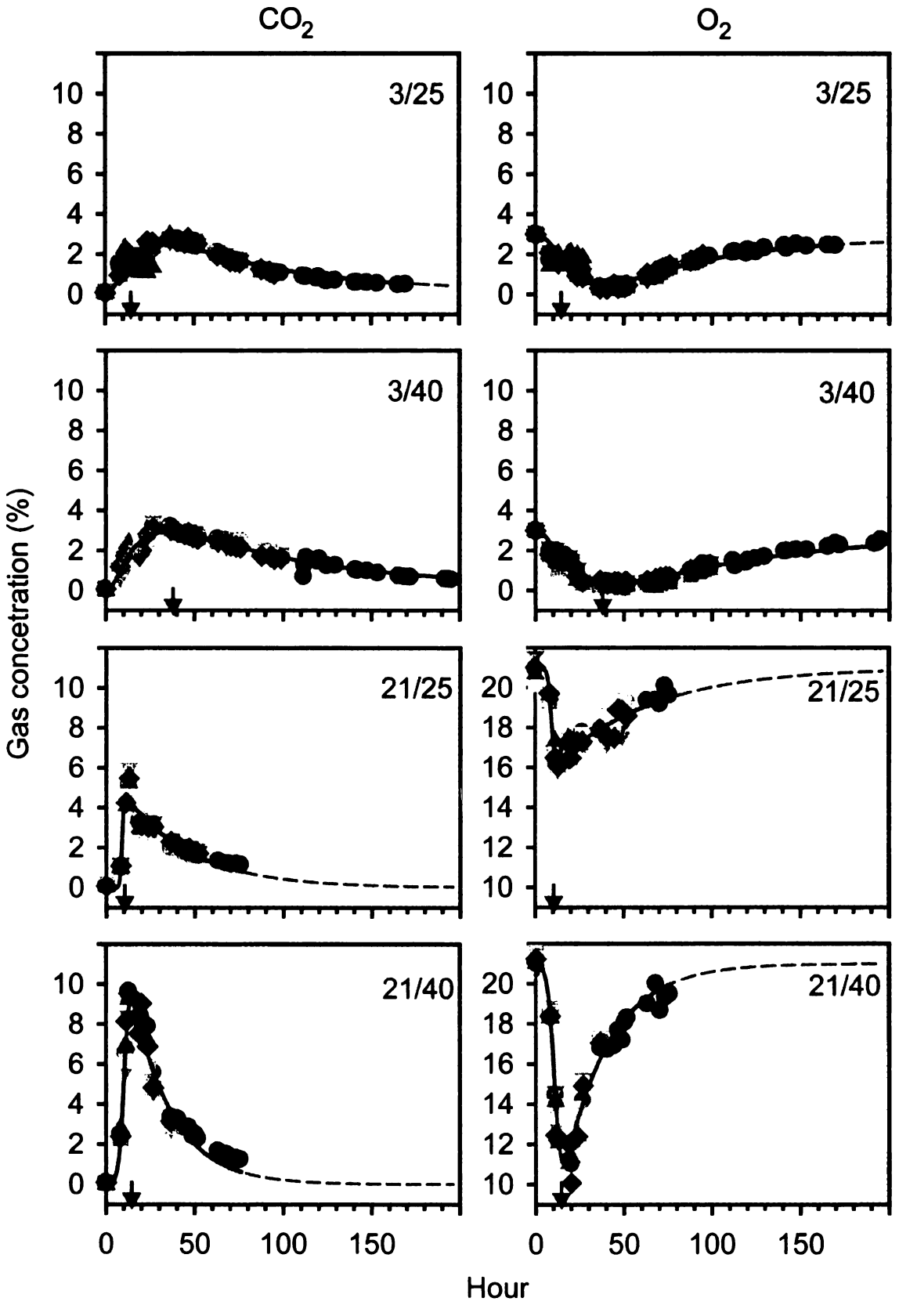
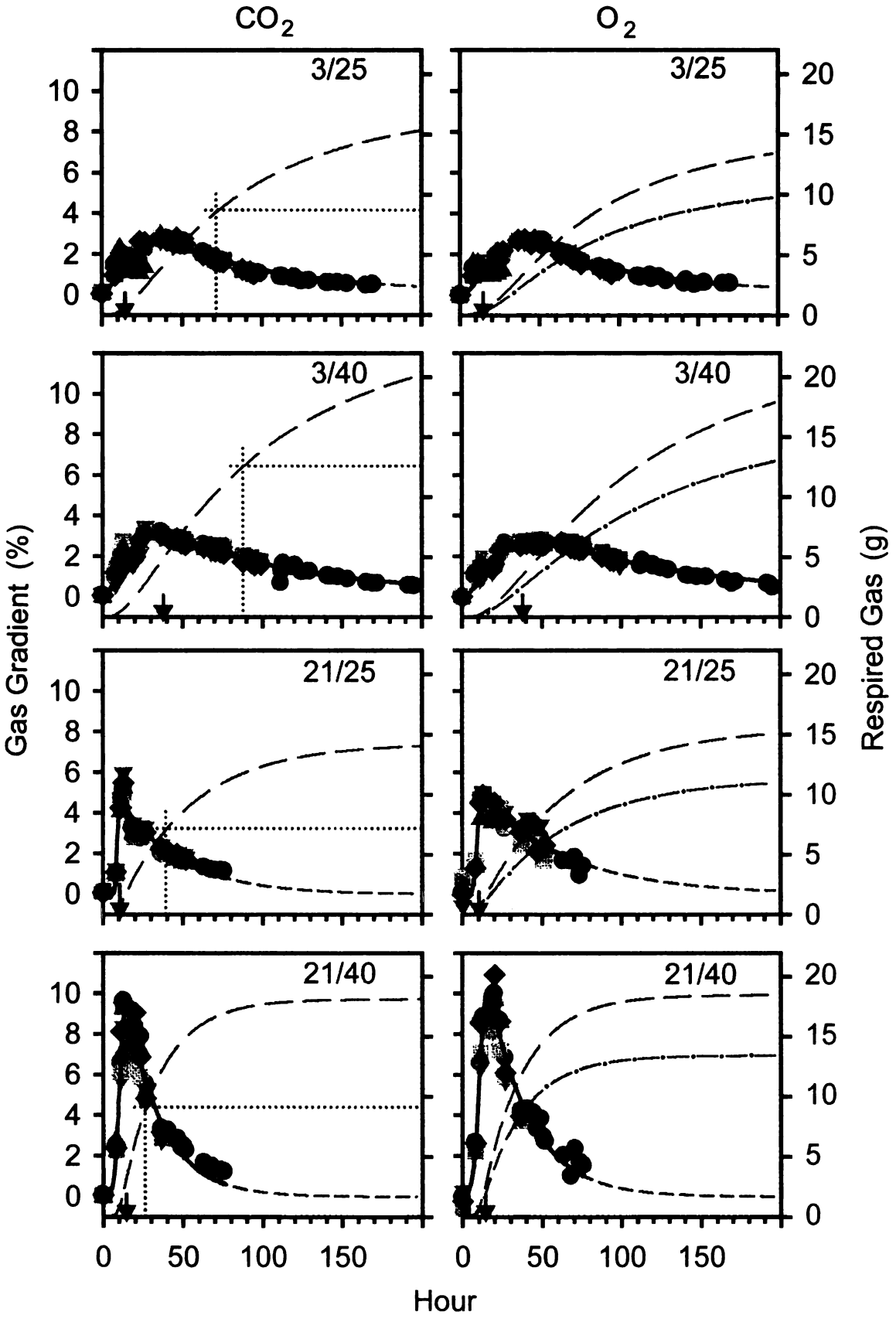
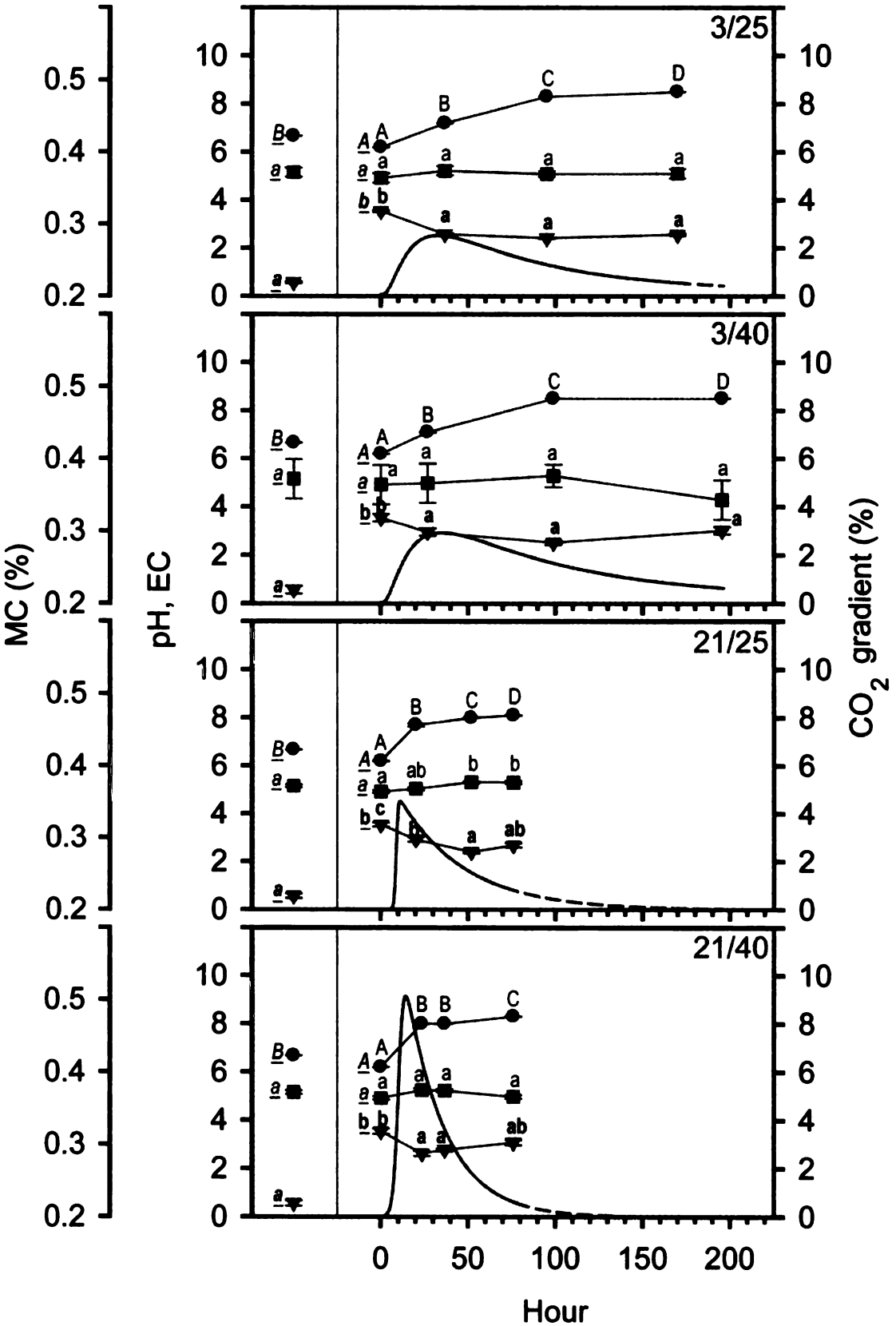

CO2
O2
3/25
3/40
21/25
21/40
Gas concetration (%)
Hour
0
50
100
150

Figure 3.2. Gas gradient (%) and respired respiratory gas (g) of amended medium under four different treatments (percent O_2/ temperature °C). Arrow indicates hour at which the target internal temperature was reached by self-heating at which point it is maintained at the target value. The bold solid line and extended dashed line is the fitted equation (#8005 or #8062 Table Curve 2D v5.01 SYSTAT, Software Inc.) based on sample data. The dashed line with dots is the accumulated consumption of oxygen (g) based on the O_2 gas gradient (%) from the media. Long dashed line is the accumulated release of carbon dioxide (g) based on either the accumulative sum of the CO_2 or O_2 gas gradient (%) equation. The fine doted line indicates the hour and value (g) at which half of the maximum CO_2 respiration was reached.


CO_2
O_2
3/25
3/40
21/25
21/40
Gas Gradient (%)
Respired Gas (g)
Hour
0
50
100
150
0
2
4
6
8
10
0
5
10
15
20

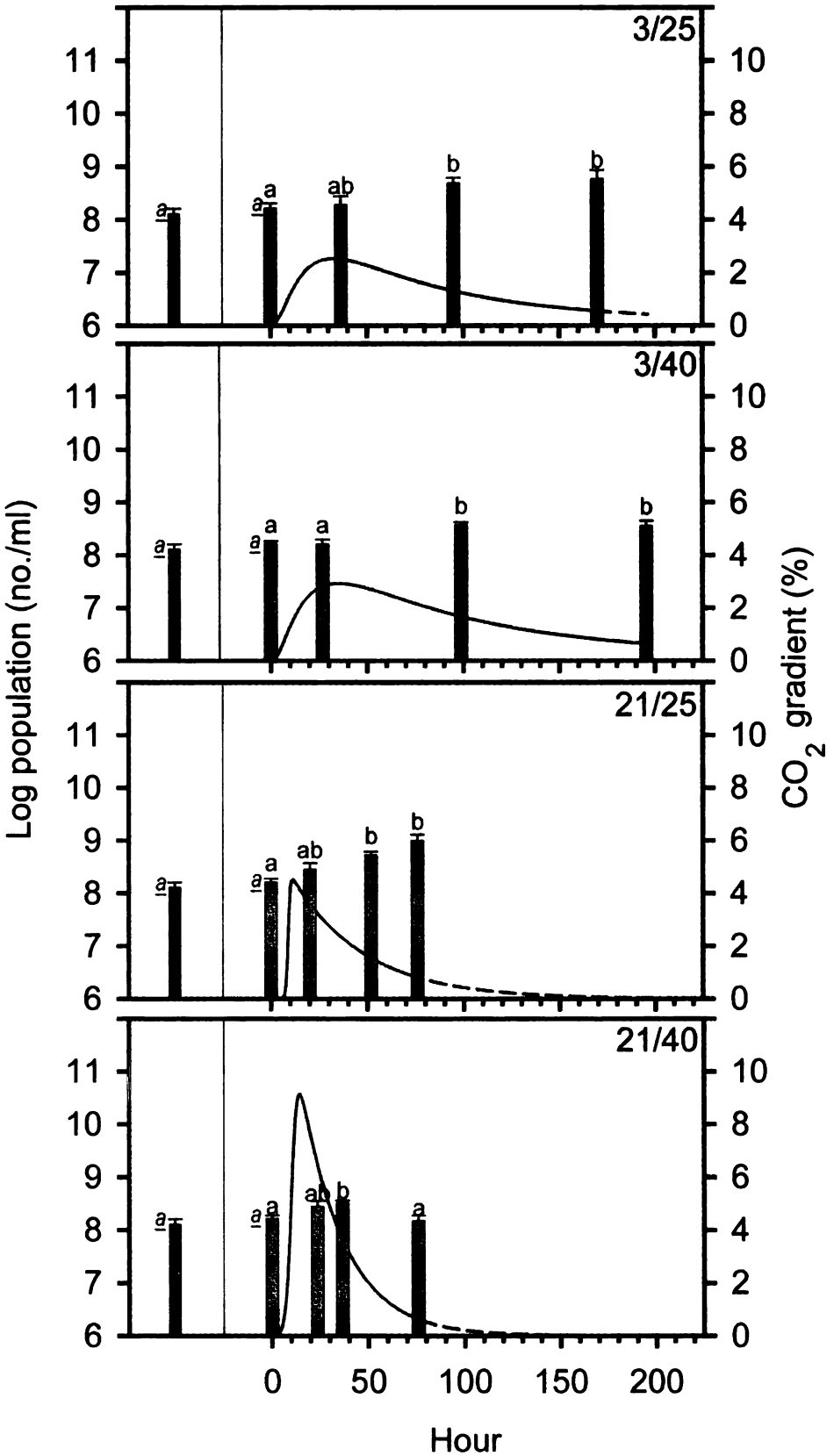
The medium pH, EC, and moisture content of the four treatments had the same trend over the experiment. There was a drop in the pH due to the amendment (6.7 to 6.2) followed by a larger increase by the first sample taken (peak CO_2) with subsequent further increases. The pH range for all four treatments was 6.2 to 8.3. The EC value underwent a sharp increase from 0.57 to 3.5 $dS \cdot m^{-1}$ due to the amendment followed by a reduction over the duration of each of the treatments. The moisture content remained stable throughout all four treatments starting with 37.2% and 36.4% respectively for the non-amended and amended compost. In all but one sample (34.3%) the range was from 36.4% to 37.6%. (Figure 3.3)

Figure 3.3. Changes in pH (circle), MC (square), and EC dS · m^{-1} (triangle) of amended medium under four different treatments. The solid and dashed lines respectively represent the calculated and projected gradient of the carbon dioxide. Graph segment without designated x axis units is non-amended medium before treatment.


3/25
3/40
21/25
21/40
MC (%)
pH, EC
CO_2 gradient (%)
Hour
0
50
100
150
200

There is no statistical difference of the total bacterial numbers between non-amended and amended medium at time 0. Under all treatments there are significant differences by the third sample interval occurring after the peak gradient of CO_2 and maintained in all but one of the treatments. The strongest possible explanation for the lack of continued exponential growth of the bacterial population (Figure 3.4) is due to predator prey relationships that exist, potentially the bacterial feeding nematode population that was enumerated in previous experiments.

Figure 3.4. Total enumerated bacterial population of an activated compost water extract. The bars are of the population over time. The solid and dashed lines respectively represent the calculated and projected gradient of the carbon dioxide. Graph segment without designated x axis units is non-amended medium before treatment.


3/25
3/40
21/25
21/40
Log population (no./ml)
CO_2 gradient (%)
Hour
0
50
100
150
200
6
7
8
9
10
11
0
2
4
6
8
10
a
ab
b

In all four treatments there is no statistically significant difference between non-amended and amended medium at time zero with respect to total fungal biomass values of 45.8 and 36.0 $\mu g \cdot mL^{-1}$. The differences in the active fungal biomass at time zero are statistically significant in three of the four treatments with the values of 6.86 (non-amended) and 1.35 $\mu g \cdot mL^{-1}$ (amended). Cause for the decrease may include increase of EC at the time of amendment addition or biological activity (bacterial) occurring in the overnight shipment to the lab.

The total fungal biomass did not undergo statistical change under the treatment conditions 3% O_2/25 °C over the duration of the experiment. There is a difference of the active fungal biomass between the amended medium (1.35 $\mu g \cdot mL^{-1}$) at P0 and that taken at P1 (8.94 $\mu g \cdot mL^{-1}$). The values of P2 and P3 (4.84 and 1.75 $\mu g \cdot mL^{-1}$) are not significantly different between one another, nor with either P0 or P1.

The total fungal biomass in the P1 sample was significantly different under the treatment conditions of 3% O_2/40 °C with a value of 68 $\mu g \cdot mL^{-1}$. The other three samples (P0, P2, and P3) were not different from one another with values ranging from 30.94 to 34.67 $\mu g \cdot mL^{-1}$. In evaluating the four samples for active fungal biomass there are no statistical difference. The trend is that there is a single significant difference between samples of total fungal biomass, but not for the active fungal biomass.

The total fungal biomass changed over time under conditions of 21% O_2 /25 °C for sample P0 and P2 with respective values of 36 and 19.41 $\mu g \cdot mL^{-1}$. There is no

statistical difference between P1 and P3 (19.41 and 30.37 $\mu g \cdot mL^{-1}$) and that of the other two (P0 and P3). The active fungi biomass is not significantly different over the duration of treatment.

The total fungal biomass changed over time under conditions of 21% O_2 /40 °C for sample P0 and P2 with respective values of 35.0 and 23.26 $\mu g \cdot mL^{-1}$. There is no statistical difference between P1 and P3 (32.95 and 20.06 $\mu g \cdot mL^{-1}$) and that of the other two (P0 and P3). The active fungal biomass is not significantly different over the duration of treatment (Figure 3.5).

For all the treatments the overall trend of the total fungal biomass is to remain constant over the duration of the treatment. In contrast, the active biomass has a significant increase between the amended medium (P0) and the first sample (P1) followed by an overall decrease to the end of the experiment.

Figure 3.5. Fungal biomass of an activated compost water extract from four treatments. The bars (solid and textured) represent the active and total biomass dynamics respectively over time. Graph segment without designated x axis units is non-amended medium. The solid and dashed lines respectively represent the calculated and projected gradient of the carbon dioxide.

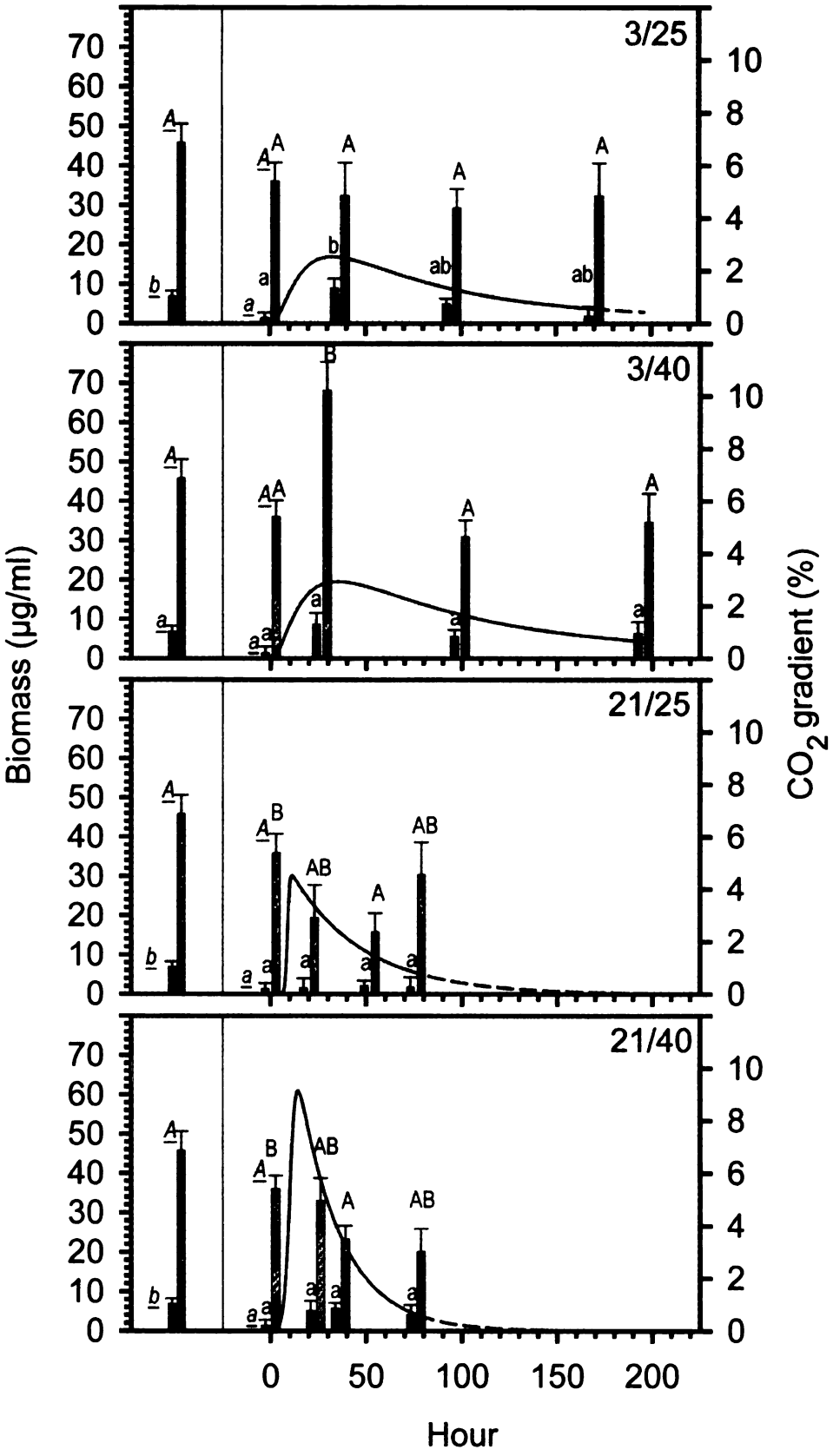

3/25
3/40
21/25
21/40
Biomass (µg/ml)
CO_2 gradient (%)
Hour
0
50
100
150
200

Table 3.2. EC and pH of a 1:10 water extraction of non-amended and amended medium.

Sample interval	Non - amended medium		Amended medium	
	EC	pH	EC	pH
P0	0.16	7.48	1	6.67

	3/25		3/40		21/25		21/40	
	EC	pH	EC	pH	EC	pH	EC	pH
P1	0.73	6.9	0.79	6.9	0.79	7	0.79	7.6
P2	0.67	8.53	0.69	8.67	0.69	7.53	0.76	8.37
P3	0.73	8.6	0.82	8.6	0.77	7.9	0.81	8.6

A tentative identification of several gas volatile compounds was made from selected ions. The values obtained for each of the five compounds across the four treatments (O_2%/Temperature °C; 3/25, 3/40, 21/25, 21/40) are represented in Figures 3.6 - 3.10.

The compound A (tentative identification as Hexane, 2,4 - dimethyl-, 3(2H)-Furanone) is an example of a compound that only evolved under the high temperature treatments. The initial detection occurred concurrently within each treatment of reaching its target temperature of 40 °C. Product levels between treatments were detected in opposing trends. Under the regulated low O_2 condition the release is an erratic increase over the duration of sampling. Under high O_2 treatment the initial peak is the highest concentration and the decline is steady and only drops sharply at the end with the last sample. As the ion was not detected in either of the O_2 treatments under which temperature was maintained at 25 °C, it may be that both elevated temperature and sufficient O_2 are necessary for the production of this compound (Figure 3.6).

The compound B (tentative identification as 3(2H)- Furanone, 4-hydroxy-5(hydroxy methyl) -2- methyl-) was detected under both the low and high target temperature (25 and 40 °C) of the regulated low O_2 treatments. A minimum of product was detected in the first sample. Maximum response occurred shortly before or near that of the maximum CO_2 production which coincides, under these treatments with O_2 levels approaching less than 0.05%. The response and duration are similar between the two treatments. Shortly after hour 48.5 the compound sharply decline and there is little to no

production for the remainder of either treatment. It is produced independent of the two temperature settings and is closely correlated with the diminished O_2 levels (Figure 3.7).

The compound C (tentative identification as Oxazole, 4,5-dihydro-2,5-dimethyl-) was detected at hour zero at low levels. The primary burst and largest response occurred within the first 24 to 30 hours in all four treatments. The treatments with higher O_2 had the greatest response at hour 16; that with lower temperate was greater. The inverse occurred under the lower O_2 regimes with the elevated temperature having a larger response. As of hour 42.5 the decline is completed and thereafter responses are at low levels with slight fluctuations for the remainder of all treatments (Figure 3.8).

The compound D (tentative identification as 3-Heptanone, 5-methyl-) was detected at low levels in first samples taken under all treatments. The low O_2 treatments produced a large response before hour 48.5. It appears that it did not coincide with either of the target temperatures being attained. Aside from a small peak at hour 65.5 under the elevated temperature with regulated low O_2 the response remained near or at zero. Under the high O_2 high treatments the response remained muted for the first 48 hours and there after was not detected (Figure 3.9).

The compound E (tentative identification as methyl disulfide) was detected in three of the four treatments with two as of hour 24, and the other as of hour 42.5. In the 21/40 at hour16 the largest response of all treatments occurred followed by a drop by

half at hour 24. At this point in time limited levels were detected in all other treatments. After hour 48.5 under treatment 3/25 the compound was not detected in contrast to all other treatments where low levels detected levels. It appears that there is a correlation between the greater response and that of the elevated temperature under the regulated high O_2 treatment (Figure 3.10).

Of the large number of ions detected a small number were selected (their tentative association with a particular compound) to demonstrate the dynamic changes within and differences between the treatments. These compounds are byproducts of either a particular or group of the microbial population under the treatment condition. Although no association is made here with these compounds and biological activity, nor the efficacy upon pathogen and host dynamics, the compounds could be used as a signature-indicator of when a specific set of conditions are met for the production of activated compost water extract.

Figure 3.6. Total ion count of compound A (tentative identification as Hexane, 24 - dimthyl-, 3(2H)- Furanone) volatized from four treatments of amended medium. The black dots represent the total ion count response of the mass spectrometer. Solid line represents the data based equation of the carbon dioxide gradient. The arrow indicates the time at which the mixture within the bioreactor self heated to the target temperature and thereafter maintained for the duration of the treatment.

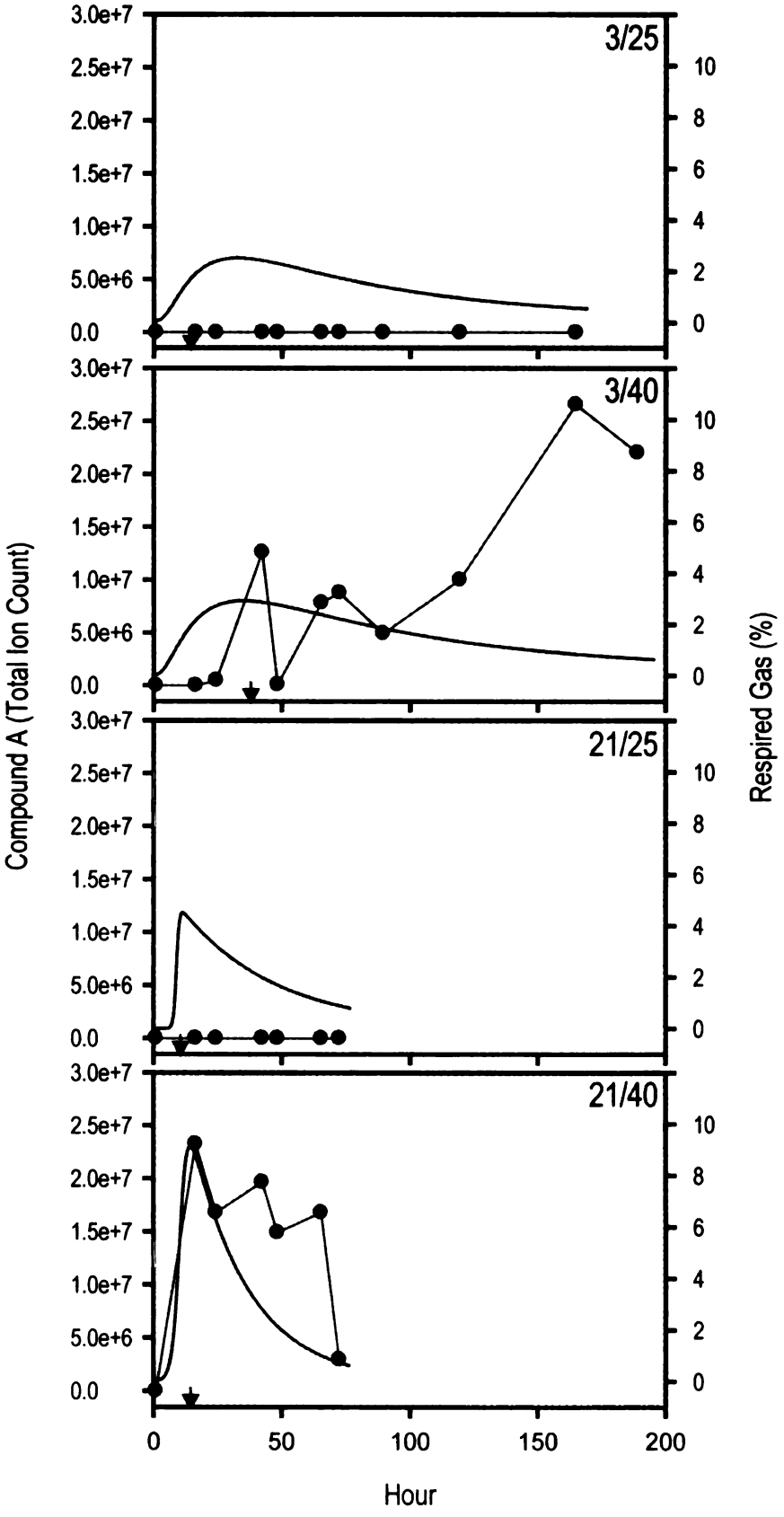

3/25
3/40
21/25
21/40
3.0e+7
2.5e+7
2.0e+7
1.5e+7
1.0e+7
5.0e+6
0.0
10
8
6
4
2
0
0
50
100
150
200
Hour
Compound A (Total Ion Count)
Respired Gas (%)

Figure 3.6. Total ion count of compound A (tentative identification as Hexane, 24 - dimthyl-, 3(2H)- Furanone) volatized from four treatments of amended medium. The black dots represent the total ion count response of the mass spectrometer. Solid line represents the data based equation of the carbon dioxide gradient. The arrow indicates the time at which the mixture within the bioreactor self heated to the target temperature and thereafter maintained for the duration of the treatment.

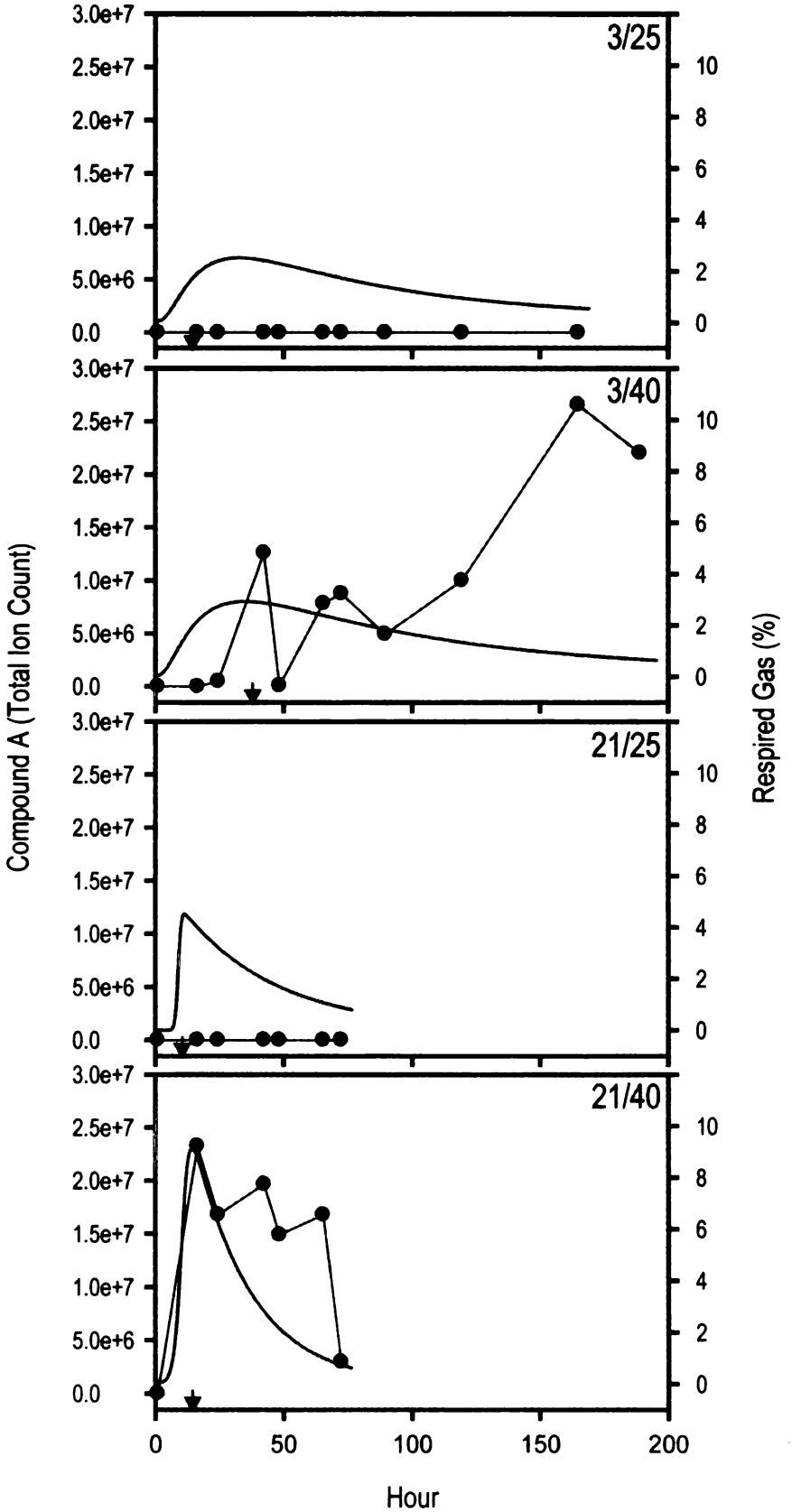

3/25
3/40
21/25
21/40
3.0e+7
2.5e+7
2.0e+7
1.5e+7
1.0e+7
5.0e+6
0.0
10
8
6
4
2
0
0
50
100
150
200
Hour
Compound A (Total Ion Count)
Respired Gas (%)

Figure 3.6. Total ion count of compound A (tentative identification as Hexane, 24 - dimthyl-, 3(2H)- Furanone) volatized from four treatments of amended medium. The black dots represent the total ion count response of the mass spectrometer. Solid line represents the data based equation of the carbon dioxide gradient. The arrow indicates the time at which the mixture within the bioreactor self heated to the target temperature and thereafter maintained for the duration of the treatment.

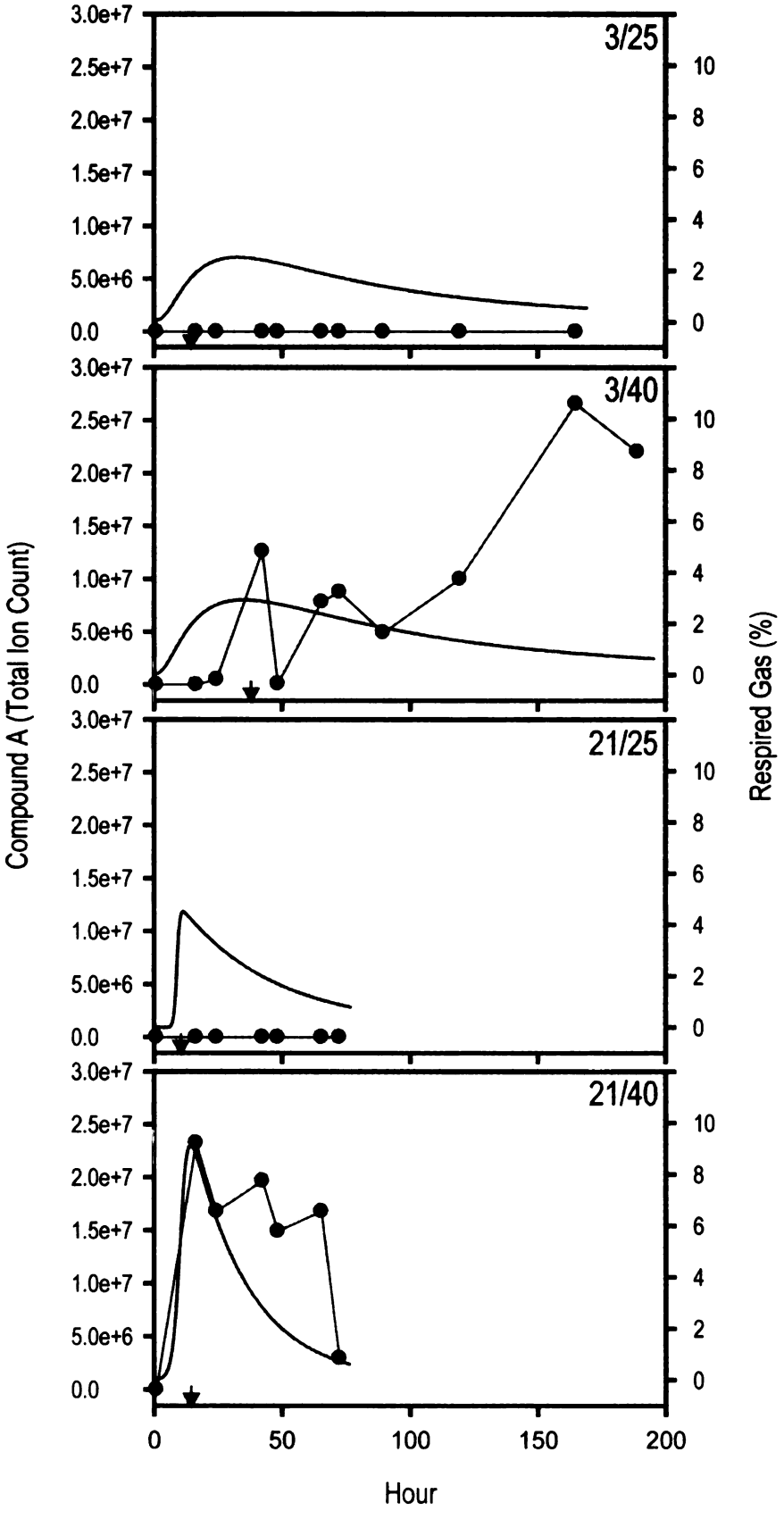

3/25
3/40
21/25
21/40
3.0e+7
2.5e+7
2.0e+7
1.5e+7
1.0e+7
5.0e+6
0.0
10
8
6
4
2
0
0
50
100
150
200
Hour
Compound A (Total Ion Count)
Respired Gas (%)

Figure 3.7. Total ion count of compound B (tentative identification as 3(2H)- Furanone, 4-hydroxy-5(hydroxy methyl) -2- methyl-) volatized from four treatments of amended medium. The black dots represent the total ion count response of the mass spectrometer. Solid line represents the data based equation of the carbon dioxide gradient. The arrow indicates the time at which the mixture within the bioreactor self heated to the target temperature and thereafter maintained for the duration of the treatment.

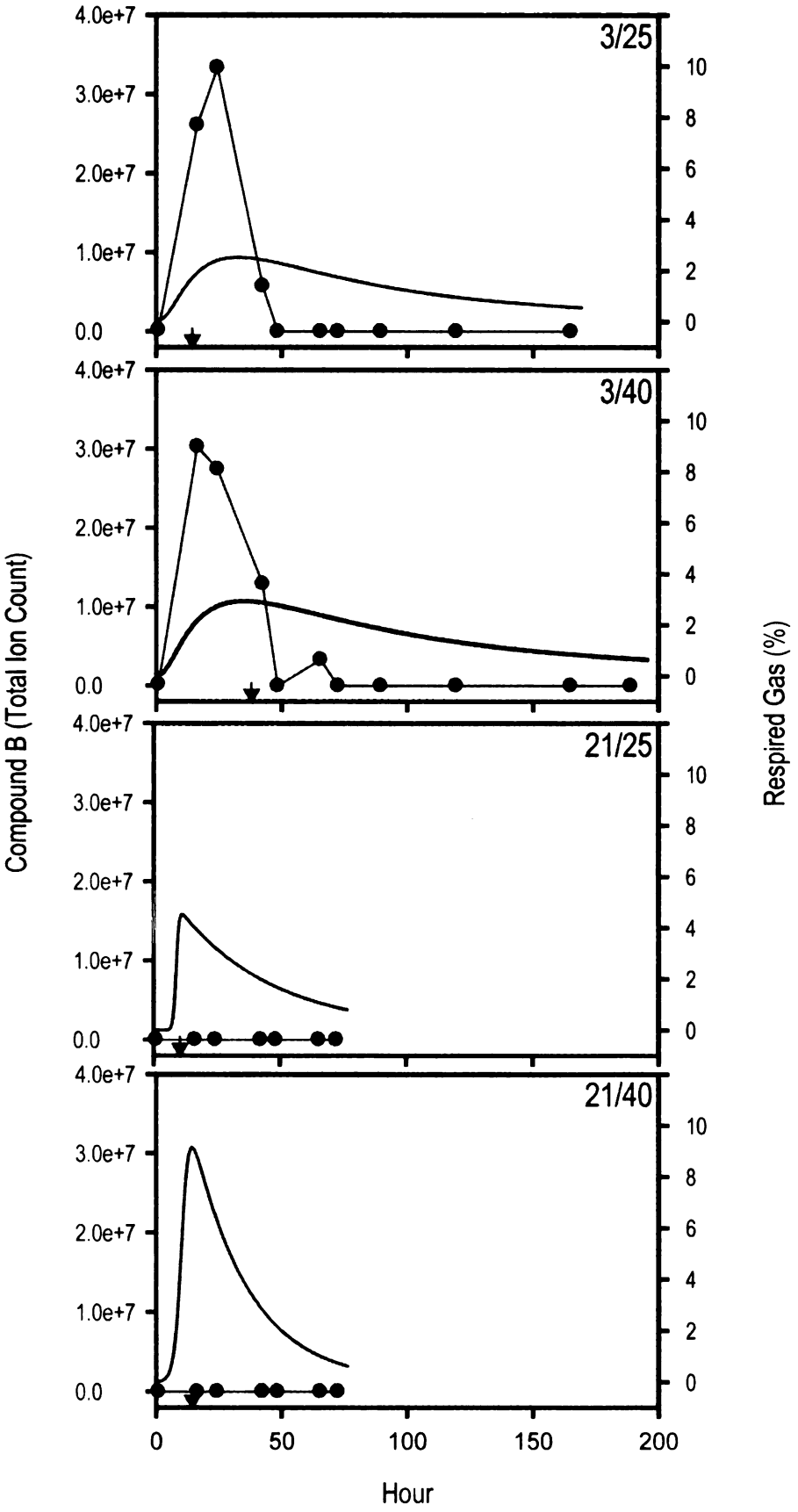

3/25
3/40
21/25
21/40
4.0e+7
3.0e+7
2.0e+7
1.0e+7
0.0
10
8
6
4
2
0
0
50
100
150
200
Hour
Compound B (Total Ion Count)
Respired Gas (%)

Figure 3.8. Total ion count compound C (tentative identification as Oxazole, 4,5-dihydro - 2,5- dimthyl -) volatized from four treatments of amended medium. The black dots represent the total ion count response of the mass spectrometer. Solid line represents the data based equation of the carbon dioxide gradient. The arrow indicates the time at which the mixture within the bioreactor self heated to the target temperature and thereafter maintained for the duration of the treatment.

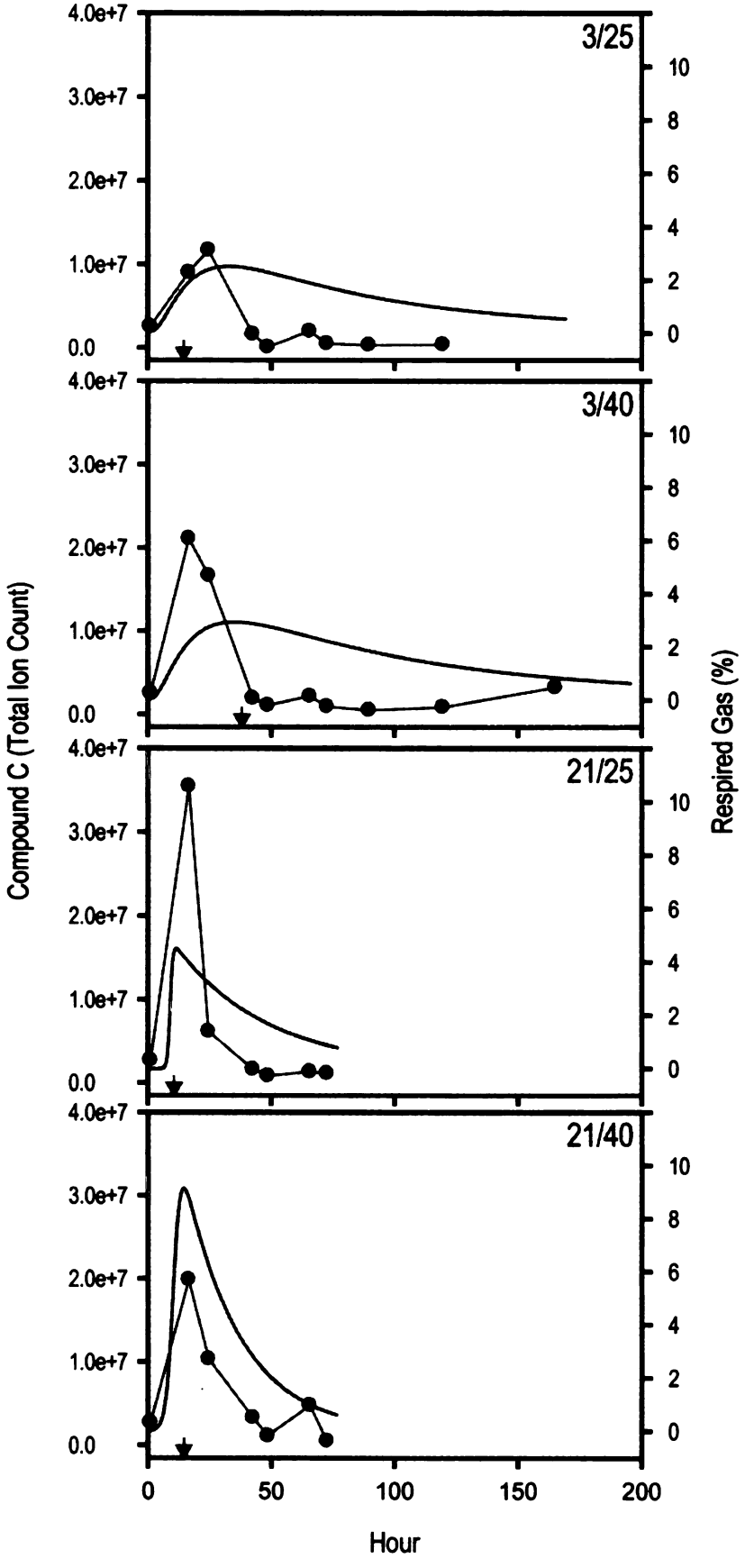

3/25
3/40
21/25
21/40
4.0e+7
3.0e+7
2.0e+7
1.0e+7
0.0
10
8
6
4
2
0
0
50
100
150
200
Hour
Compound C (Total Ion Count)
Respired Gas (%)

Figure 3.9. Total ion count of compound D (tentative identification as 3-Heptanone, 5-methyl-) volatized from four treatments of amended medium. The black dots represent the total ion count response of the mass spectrometer. Solid line represents the data based equation of the carbon dioxide gradient. The arrow indicates the time at which the mixture within the bioreactor self heated to the target temperature and thereafter maintained for the duration of the treatment.

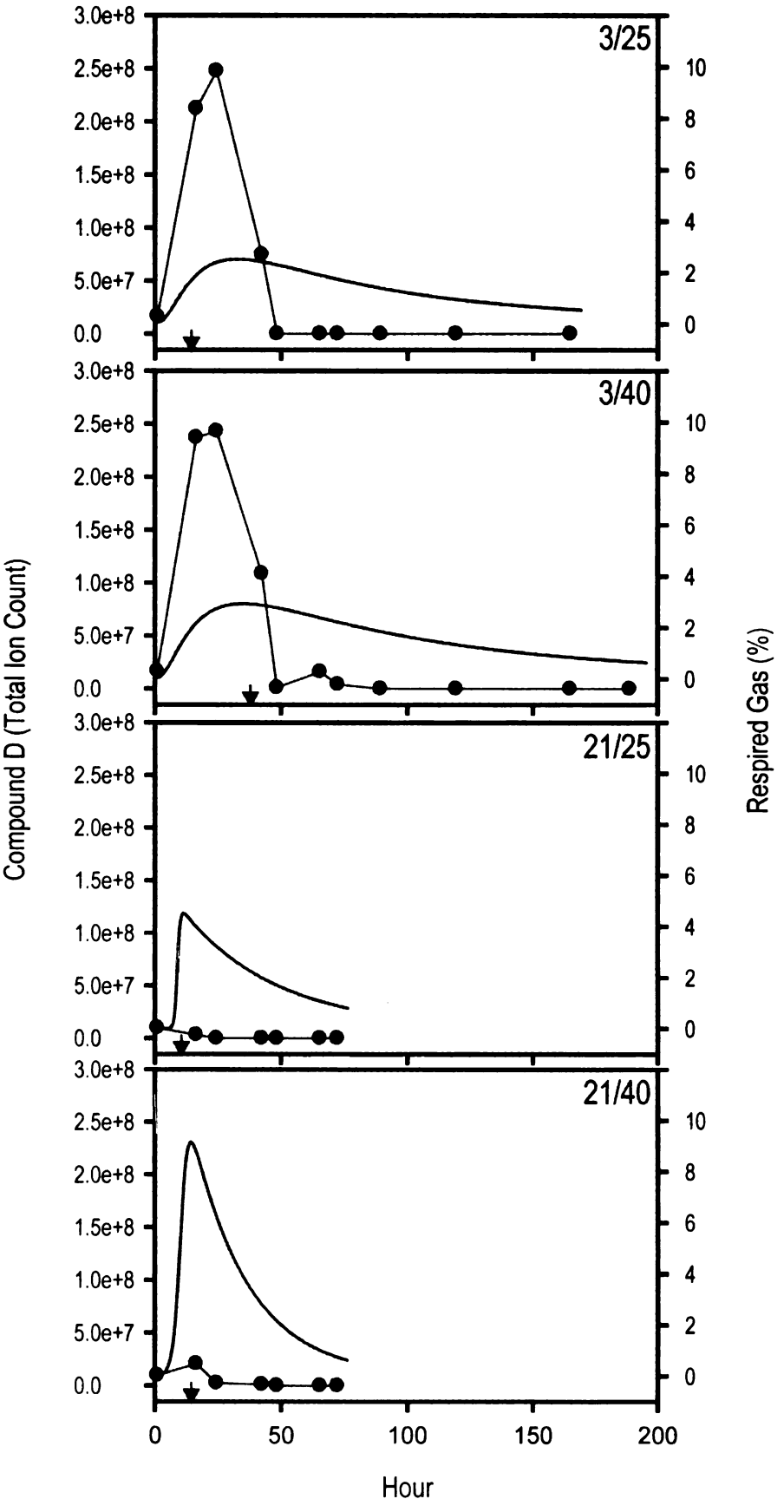
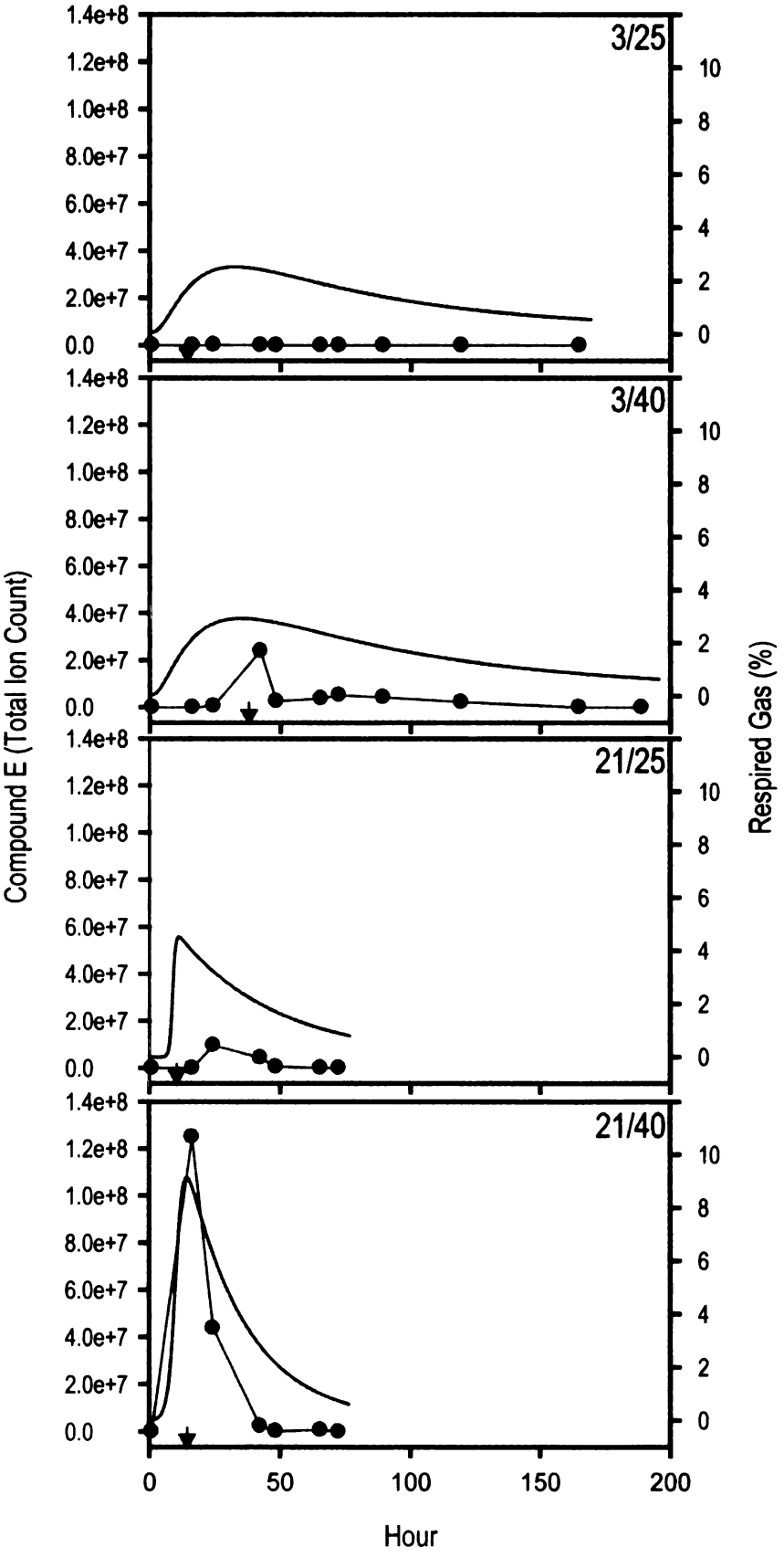

3/25
3/40
21/25
21/40
3.0e+8
2.5e+8
2.0e+8
1.5e+8
1.0e+8
5.0e+7
0.0
10
8
6
4
2
0
0
50
100
150
200
Hour
Compound D (Total Ion Count)
Respired Gas (%)

Figure 3.10. Total ion count of compound E (tentative identification as Methyl disulfide (or Disulfide, dimthyl) volatized from four treatments of amended medium. The black dots represent the total ion count response of the mass spectrometer. Solid line represents the data based equation of the carbon dioxide gradient. The arrow indicates the time at which the mixture within the bioreactor self heated to the target temperature and thereafter maintained for the duration of the treatment.


3/25
3/40
21/25
21/40
Compound E (Total Ion Count)
Respired Gas (%)
Hour
1.4e+8
1.2e+8
1.0e+8
8.0e+7
6.0e+7
4.0e+7
2.0e+7
0.0
10
8
6
4
2
0
0
50
100
150
200

Characterization of the bacterial population was done using T-RFLP. The treatment with low O_2 and elevated temperature (3/40) had 46 fragment lengths (bases) identified from the amended medium with a height greater than 50 in at least one of the four samples. Of those identified, 26 base pairs meet the 125bp height selection criteria, of which, 20 are transient, 1 persistent-stable, and 5 are persistent-non-stable. The treatment with high O_2 and elevated temperature (21/40) had 62 fragment lengths (bases) identified with at height greater than 50 in at least one of the three samples and 36 meet the 125bp height selection criteria of which, 18 are transient, 8 persistent-stable, and 10 persistent-non-stable (Figures 3.11 – 3.14). The r^2 value for the identified peaks from the technical replicate of the treatments (3/40 and 21/40), within each phase ranges from 0.91 to 0.99 and 0.92 to 0.97 respectably. The r^2 value across all phases and the base pairs within each treatment is 0.90 and 0.99 respectively (Table 3.3).

Table 3.3. Inter and intra values from technical replicates 1 and 2 taken at various phases of two treatments of amended medium.

Treatment: 3% O_2 / 40 °C

Identified peaks

Phase	r^2	slope equation
0	0.917	y = 0.8675x + 2.3141
1	0.992	y = 1.118x - 3.0938
2	0.985	y = 0.5583x + 15.848
3	0.992	y = 0.8066x + 9.6459
0-3	0.901	y = 0.7121x + 22.815

Selected peaks (height > 125)

Phase	r^2	slope equation
0	0.895	y = 0.8712x + 1.259
1	0.990	y = 1.1131x + 0.0428
2	0.984	y = 0.5576x + 17.109
3	0.990	y = 0.8043x + 11.594
0-3	0.885	y = 0.6978x + 35.884

Treatment: 21% O_2 / 40 °C

Identified peaks

Phase	r^2	slope equation
0	0.923	y = 0.8658x + 3.9012
1	-	-
2	0.995	y = 0.8544x - 3.1483
3	0.974	y = 0.9548x - 2.5726
0, 2, 3	0.987	y = 0.8607x + 3.6711

Selected peaks (height > 125)

Phase	r^2	slope equation
0	0.911	y = 0.8716x + 1.7784
1	-	-
2	0.998	y = 0.8575x - 4.4444
3	0.969	y = 0.9633x - 6.64
0, 2, 3	0.989	y = 0.8625x + 3.9378

Figure 3.11. Average of two technical replicates of identified fragment length (bases) from amended medium in a bioreactor with O_2 levels no greater than 3% and temperate not exceeding 40 °C after self heating. The dashed line is the 125 fluorescent height Inserted graph is based on the intra values of the sampling phases.

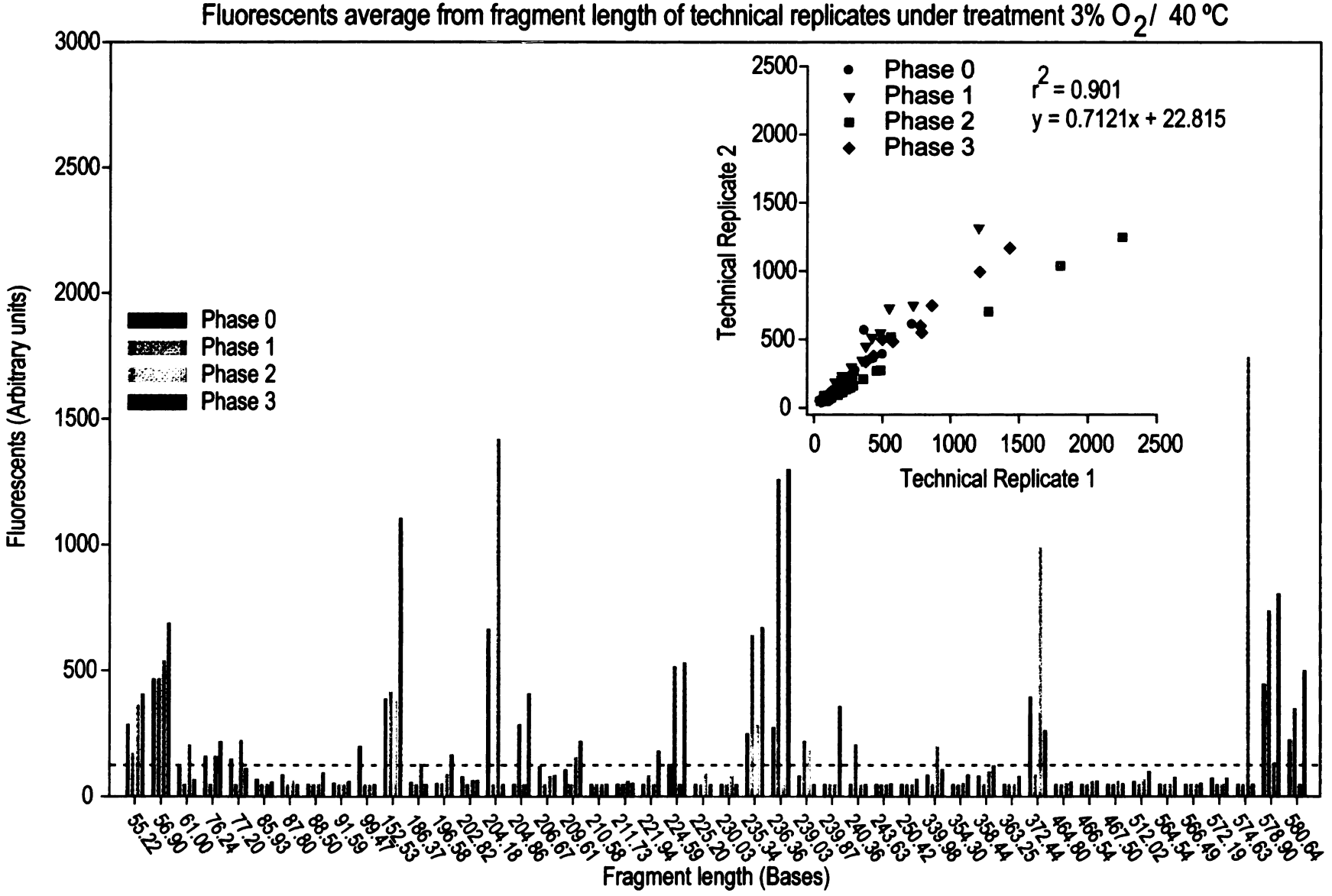

Fluorescents average from fragment length of technical replicates under treatment 3% O2/ 40 °C
Fluorescents (Arbitrary units)
Fragment length (Bases)
Phase 0
Phase 1
Phase 2
Phase 3
r2 = 0.901
y = 0.7121x + 22.815
Technical Replicate 2
Technical Replicate 1
55.22
56.90
61.00
76.24
77.20
85.93
87.80
88.50
91.59
99.47
152.53
186.37
196.58
202.82
204.16
204.86
206.67
209.61
210.58
211.73
221.94
224.59
225.20
230.03
235.34
236.36
239.03
239.87
240.36
243.63
250.42
339.98
354.30
358.44
363.25
372.44
464.80
466.54
467.50
512.02
564.54
566.49
572.19
574.63
578.90
580.64

Figure 3.12. Average fluorescent height of two technical replicates of selected fragment length from amended medium in a bioreactor with O_2 levels no greater than 3% and temperate not exceeding 40 °C after self heating. Inserted graph is based on the intra values of the sampling phases.

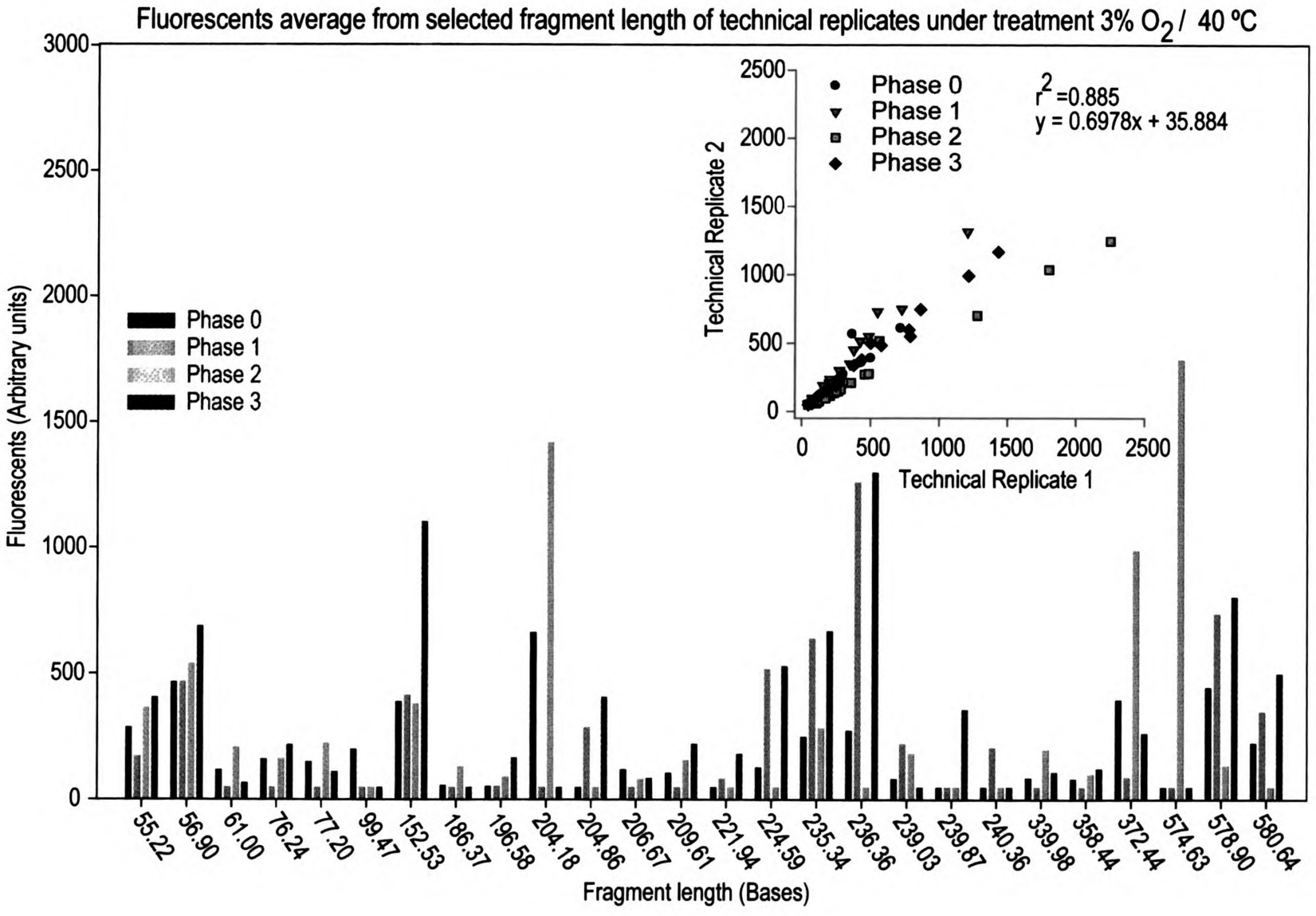

Fluorescents average from selected fragment length of technical replicates under treatment 3% O_2 / 40 ºC
Fluorescents (Arbitrary units)
Fragment length (Bases)
Phase 0
Phase 1
Phase 2
Phase 3
55.22
56.90
61.00
76.24
77.20
99.47
152.53
186.37
196.58
204.18
204.86
206.67
209.61
221.94
224.59
235.34
236.36
239.03
239.87
240.36
339.98
358.44
372.44
574.63
578.90
580.64
Technical Replicate 2
Technical Replicate 1
r^2 =0.885
y = 0.6978x + 35.884

Figure 3.13. Average fluorescent height of two technical replicates of identified fragment length (Bases) from amended medium in a bioreactor with O_2 levels no greater than 21% and temperate not exceeding 40 °C after self heating. The dashed line is the 125 fluorescents height. Inserted graph is based on the intra values of the sampling phases.

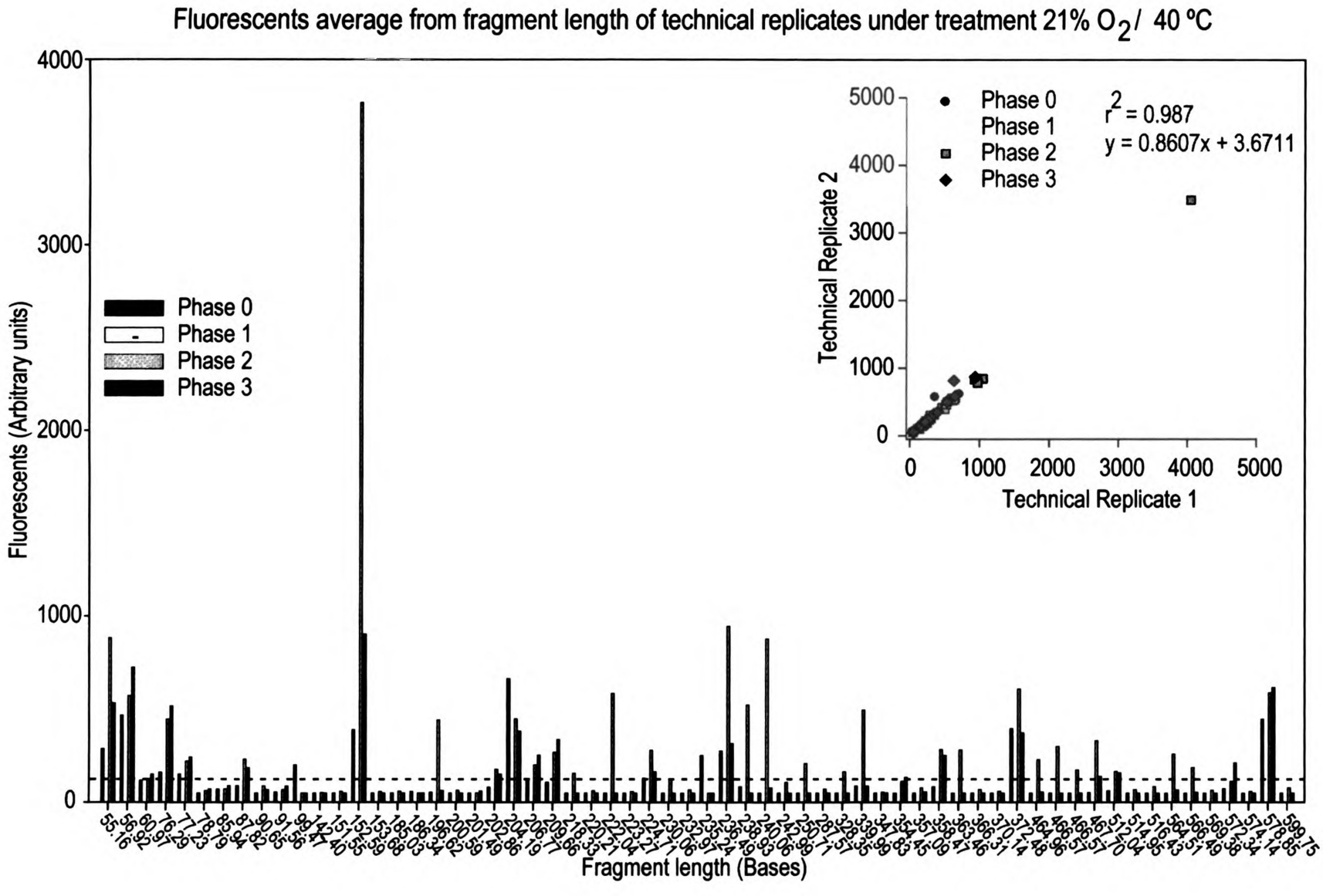
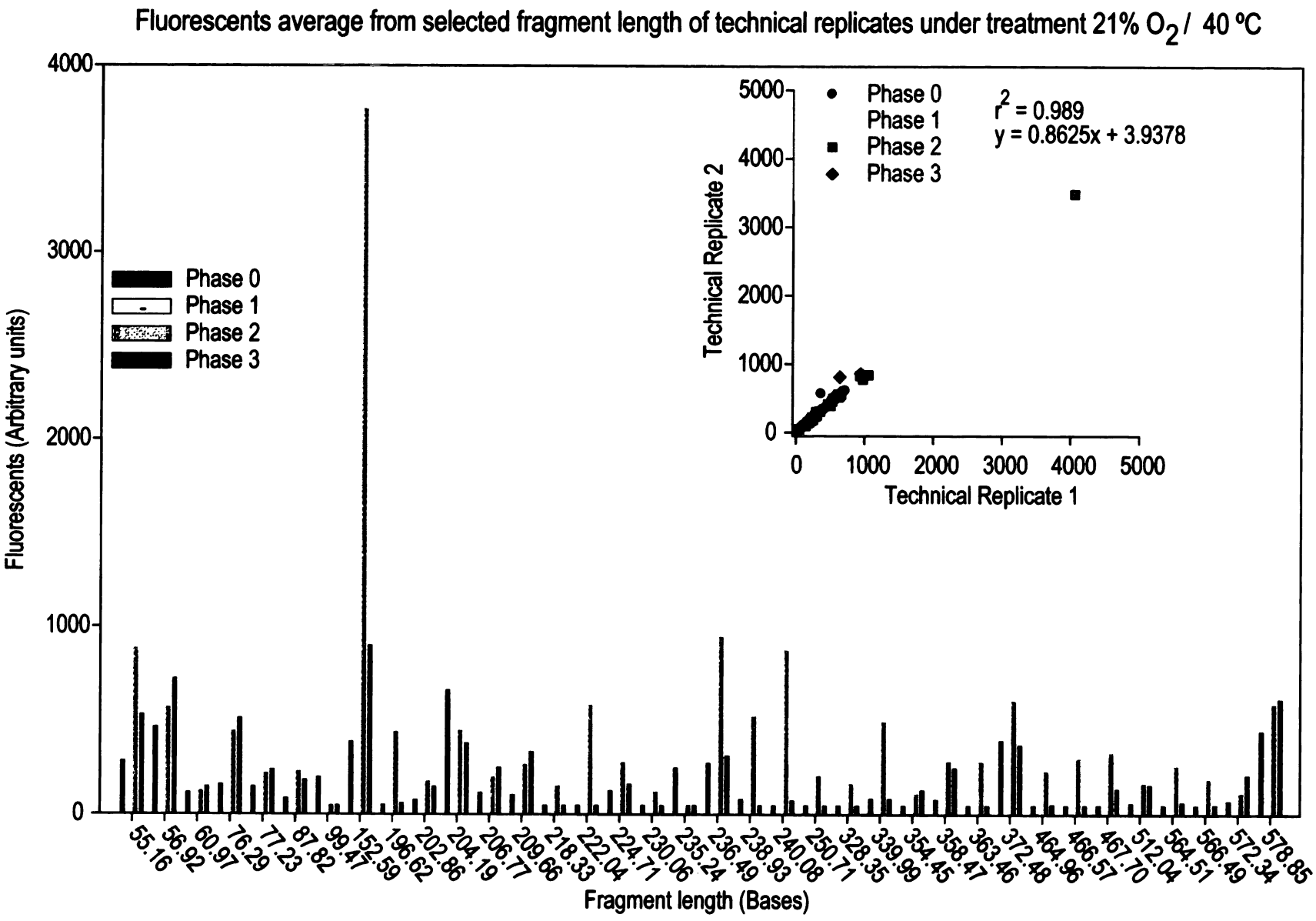

Fluorescents average from fragment length of technical replicates under treatment 21% O_2 / 40 ºC
Fluorescents (Arbitrary units)
Fragment length (Bases)
Phase 0
Phase 1
Phase 2
Phase 3
Technical Replicate 2
Technical Replicate 1
r^2 = 0.987
y = 0.8607x + 3.6711

Figure 3.14. Average fluorescent height of two technical replicates of selected fragment length (bases) from amended medium in a bioreactor with O_2 levels no greater than 21% and temperate not exceeding 40 °C after self heating. Inserted graph is based on the intra values of the sampling phases.


Fluorescents average from selected fragment length of technical replicates under treatment 21% O_2 / 40 °C
Fluorescents (Arbitrary units)
4000
3000
2000
1000
0
Phase 0
Phase 1
Phase 2
Phase 3
55.16
56.92
60.97
76.29
77.23
87.82
99.47
152.59
196.62
202.86
204.19
206.77
209.66
218.33
222.04
224.71
230.06
235.24
236.49
238.93
240.08
250.71
328.35
339.99
354.45
358.47
363.46
372.48
464.96
466.57
467.70
512.04
564.51
566.49
572.34
578.85
Fragment length (Bases)
Technical Replicate 2
5000
4000
3000
2000
1000
0
Phase 0
Phase 1
Phase 2
Phase 3
r^2 = 0.989
y = 0.8625x + 3.9378
0
1000
2000
3000
4000
5000
Technical Replicate 1

Phylogenetic inference in evolutionary trees of the bacterial population using the T-RFLP data was done with assistance by Dr. Terrance Marsh (Michigan State University). Technical replicates from all samples were grouped together based on the two enzymes (Hha I or Msp I) used in the T-RFLP procedure. The phylogenic tree using Hha I infers the least similarity from the amended medium at P0 (time zero) from that of 21/40 P2. Becoming more similar from 21/40 P2 to P0 the sequential change is 3/40 P1, P2, P3, and 21/40 P3 (Figure 3.15). The phylogenic tree using Msp I infers differentiation into two main groups. The first group, including P0 has, in order of increasing similarity, 3/40 P2 and 21/40 P3. The second has 3/40 P1 and P3 as equally least similar to P0, followed by 21/40 P2 (Figure 3.16).

Figure 3.15. The identified base pairs using Hhal 1 from treatments 3/40 (P0, P1, P2, and P3) and 21/40 (P0, P2, and P3) in an evolutionary tree based on presence/absence with unweighted pair group method with arithmetic mean (UPGMA) using phylogenetic analysis using parsimony (PAUP 4.0 Beta, David L. Swofford, Florida State University).

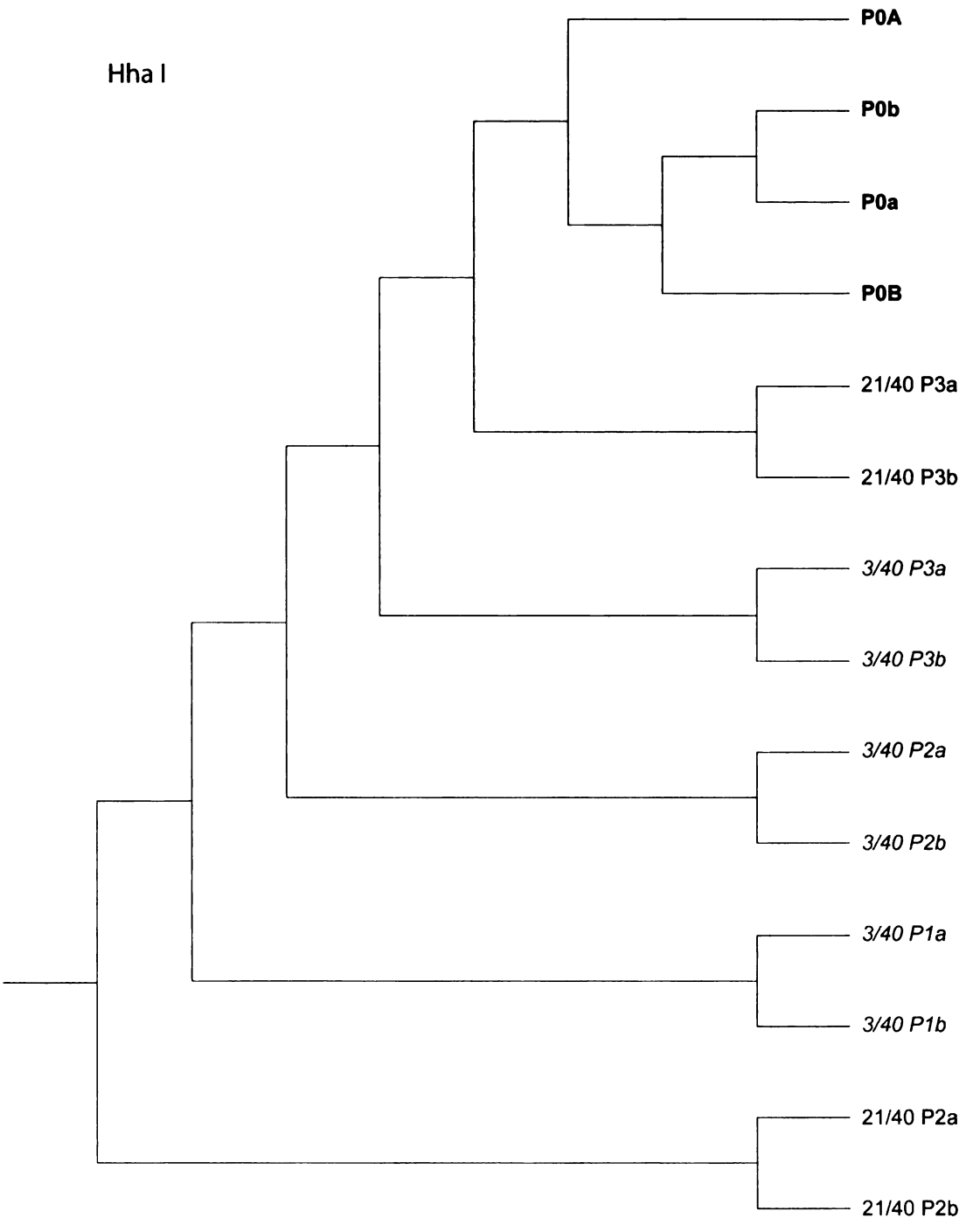
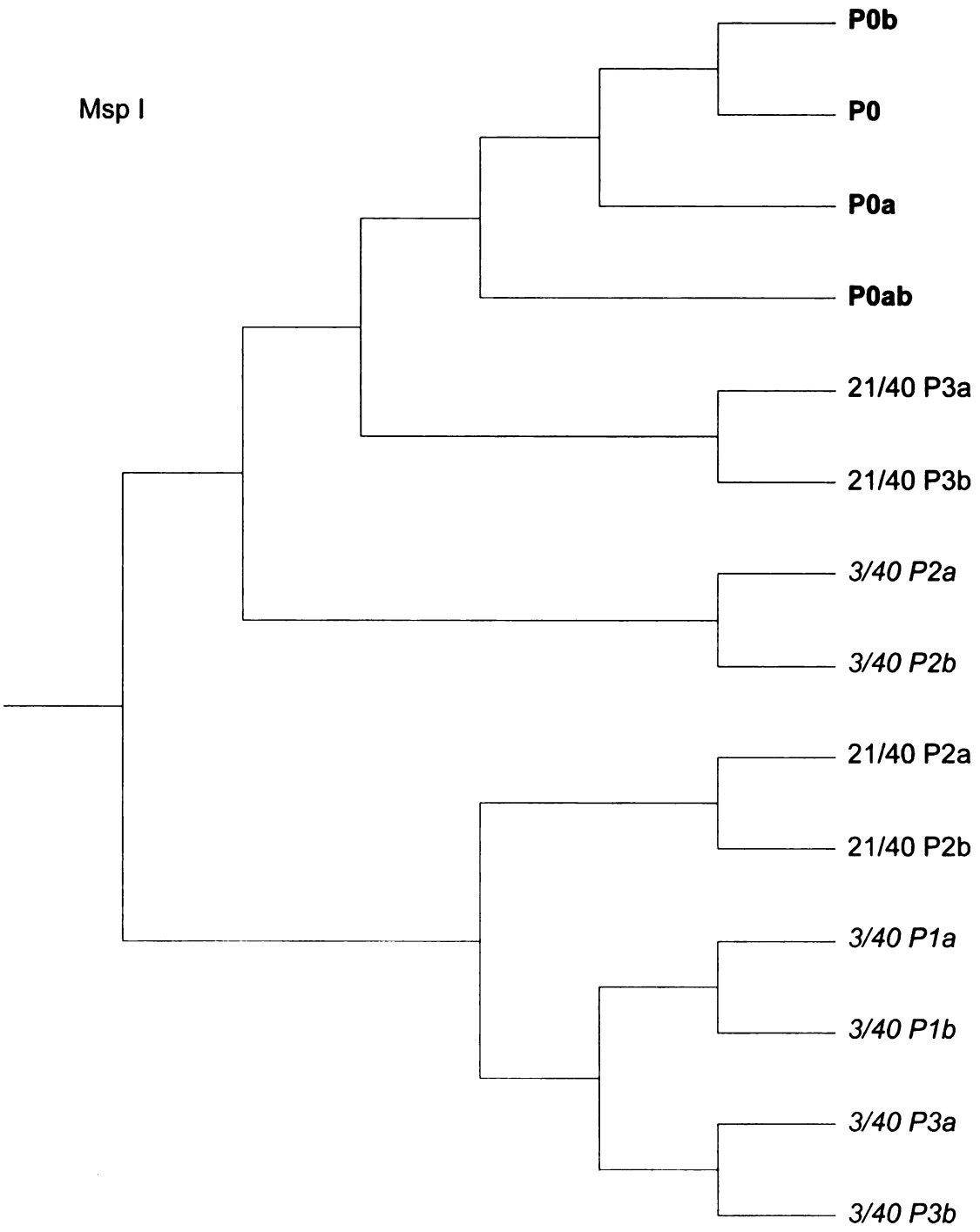

Hha I
P0A
P0b
P0a
P0B
21/40 P3a
21/40 P3b
3/40 P3a
3/40 P3b
3/40 P2a
3/40 P2b
3/40 P1a
3/40 P1b
21/40 P2a
21/40 P2b

Figure 3.16. The identified base pairs using Msp 1 from treatments 3/40 (P0, P1, P2, and P3) and 21/40 (P0, P2, and P3) in an evolutionary tree based on presence/absence with unweighted pair group method with arithmetic mean (UPGMA) using phylogenetic analysis using parsimony (PAUP 4.0 Beta, David L. Swofford, Florida State University).


Msp I
P0b
P0
P0a
P0ab
21/40 P3a
21/40 P3b
3/40 P2a
3/40 P2b
21/40 P2a
21/40 P2b
3/40 P1a
3/40 P1b
3/40 P3a
3/40 P3b

Synthesis and integration of data sets

The mitigation of specific host pathogen relationships as the result of application of compost based preparations has been identified possibly as being a combination of both abiotic and biotic factors, produced under either aerobic or anaerobic conditions. The low cost solid state bioreactor developed allowed management of gas inflow rate and O_2%, temperature, moisture content, and monitoring of CO_2 and O_2 exit gas evolution and temperature. A mature compost amended with an alfalfa based fertilizer (Bradfield) was incubated in at two oxygen (<3 or 21%) and two temperature (25 or 40 °C) levels within the bioreactors. The objective is to characterize microbial growth and population shifts and to produce a water extract to improve soil or plant health.

Under the defined conditions the changes occurring within the heterotrophic amended medium are within anticipated parameters (EC, pH, moisture content, etc.) found within composting. The results are consistent with previously conducted experiments (Chapter 2) with the same materials and protocol.

Total bacteria counts under all four treatments increased. In contrast to exponential growth of pure culture it is anticipated that reduced rate of growth was due to either predatory nematodes or different bacterial groups were able to make use of the preceding populations' biomass as conditions shifted within the medium.

Over the duration of the experiment the respective active and total fungal biomass did have significant changes in one and three of the four treatments. Unknown is the contribution of the overnight transportation for analysis upon the fungal biomass as it was not fixed with a preservative suspending all further development. Possible

contributing factors, if they indeed influenced the results, to consider are, time, O_2 treatment, sum respiration, and metabolic byproducts during the transport.

Based upon the data collected from the exit gas and the fitted equations, treatments 3/25 and 3/40 were oxygen limited for some duration during treatment which are environmental conditions different from the treatments 21/25 and 25/40. Treatment 3/25 by the time target temperature was reached (14.5 h) was oxygen limited with 1.8% O_2 as it was five hours later than when treatment 21/25 reached the target temperature (10.5 h) with 17.0% O_2. When treatment 21/25 reached the target temperature (10.5 h) the exit O_2 of treatment 3/25 was 2.3% at which point oxygen had became a limiting factor in the generation of heat as a byproduct. Some time between 0 and 10.5 h during which the exit O_2 was reduced from 3 to 2.3% the reduction in the potential heat that could be generated. Treatments 3/25 and 3/40 are below 2.3% O_2 beginning at 10.5 and 8.5 h and remain until 150 and 213 h respectively for a total of 140 h where the two treatments are below 2.3% O_2. The treatments 3/25 and 3/40 are below 1% O_2 starting from 23 and 20.4 h and remained below until 65.45 and 90.1 h respectively where both treatments are simultaneously below 1% for a total of 27 h. For the duration of the 27 h the environment, based on O_2 levels alone, is conducive for the microbial populations to be greater in similarity when compared to either of the high O_2 treatments.

Treatments 3/25 and 3/40 reached their respective target temperature at 14.5 and 38 h and the 15 °C difference is an influence upon the microbial population that may

lead to a succession shift as the temperature increase over the 23.5 h. Under treatment 3/40 half (7.5 °C) of the total temperature increase (15 °C) must have occurred between 14.5 – 26 h. Based on the fitted equation starting at 13 h the eight hour interval and O_2 concentration (%) for treatment 3/40 are respectively (0, 3); 13, 1.7; 21, 0.9; 29, 0.4; and 37, 0.3 which is a reduction over each of the eight hour intervals of 0.8, 0.5, 0.1% O_2. This decrease of O_2 directly influences potential of heat production as a metabolic byproduct implying that the larger part of the 15 °C would have occurred by 21 h. Under elevated temperature conditions some microbial groups will be able to become more dominate leading to a succession of the population. If differences were to be established they might be maintained until either 65 (>1%) or 150 h (>2.36%) when treatment 3/25 has a different O_2 concentration.

Treatment 21/25 reached target temperature at 17% O_2 and remained below for 12.5 h and treatment 21/40 reached the target temperature at 11.4% O_2. There is a 4 h difference between the time the target temperatures of 25 and 40 °C were reached indicating that the onset and any succession of the microbial population would be rapid. Based on other results (Jost, unpublished) with 21% O_2 the temperature within the bioreactor continued to increase to a maximum of 69-71 °C.

The same amendment alfalfa based fertilizer (Bradfield Organics®, Springfield Michigan; Alfalfa (3-1-5), sulfate of potash, molasses, poultry protein, humate, C:N 1:13, ash 15%) used in these experiments was analyzed and reported by Agehara and Warncke (2005)to have a total carbon and lignin (40% carbon) content of 39.9% and

9.29% (dry basis – 105 °C). Based on these values the estimated quantity of alfalfa carbon added from the 21.6 grams of amendment was 13.68 g (dry weight). From the maximum CO_2 respired data (Table 3.1 (4.83, 6.83, 3.21, and 4.62 g)) for each treatment, the percent grams carbon respired from total amendment carbon (8.6 g) as a function of treatment is 56, 79, 37, and 54% for 3/25, 3/40, 21/25, and 21/40. The temperature difference of 25 and 40 °C resulted in increased CO_2 respiration by 41 and 44% respectively for O_2 treatment of 3 and 21%. The difference of 21 and 3% O_2 resulted in increased CO_2 respiration by 50 and 49% respectively for temperature treatments at 25 and 40 °C. Therefore, the effect of increased temperature was consistent across O_2 and the effect of decreased O_2 was consistent across temperature. Of the four treatments under which each of microbial populations are metabolizing carbon under different conditions, 21/25 and 3/40 had the least and most carbon respired respectively.

An additional contributing factor to microbial population succession over time is that the amendment has a range of complexity from readily available to nearly non available carbon. Van Veen and Paul (1981) developed a simple computer simulation model based on three carbon fractions from complex substrate decomposition in soil, such as crop residue, which they identified as C1 carbohydrates and proteins, C2 cellulose and hemicellulose, and C3 lignin. The model is used to place values over time of the remaining total carbon fractions as carbon changes between the actual plant carbon (C1, C2, and C3) and the microbial products of biomass, metabolites, and released carbon in the form of CO_2. From the composts reported by Moubasher et al.

(1984) broad bean straw values from the starting and remaining percentage of the three fractions after 215 d composting, the respective available fraction from the alfalfa within the fertilizer are 6.58, 19.94, and 0.68% or 0.90, 2.73, and 0.09 g. The difference between the total and available provide the remaining (recalcitrant) respective fractions of 3.42, 6.06, and 8.42% or 0.47, 0.83, and 1.15 g. Estimates for the alfalfa amendment in the study reported here for the sum of hemicellulose, cellulose, and lignin that is available for microbial development is 27.20% or 3.72 g and that which is recalcitrant is 17.90% or 2.45 g respectively (Not calculated is the contribution of the molasses and poultry). Based on the model by Van Veen and Paul (1981) when 50% of the total carbon is remaining, the available C1 and C2 carbon has been removed from the plant material and either left the system or is in the microbial biomass. The C3 (recalcitrant C1, C2, and lignin) fraction has been reduced 2%. Of the 50% total remaining carbon, the actual plant carbon and microbial products are 21 and 26% respectively (3% unaccounted).

The availability of carbon from the three fractions changes over time so that there is a succession of microbial population from those that have a high reproduction rate using the readily available forms of carbon (r-strategists) to those that adept for optimal utilization of environmental resources (K-strategists). The availability of carbon over time contributes further to the contributing factors of oxygen and/or temperature upon microbial successions. The hour of half maximum of the 3% O_2 treatments of 25 and 40 °C is at 74 and 89 h. The additional 15 h under 3/40 conditions could be the result of different microbes or as a result some C1 and C2 materials that are allocated to the recalcitrant fraction at 25 °C become available at 40 °C. Either or both of these

explanations may be correct as the total potential maximum CO_2 released is greater under the higher temperature treatment. The hour of half maximum of the 21% O_2 treatments of 25 and 40 °C is at 40 and 27 h respectively. In contrast to the 3% O_2 treatments, the higher temperature treatment in comparison to the lower, reduced the time to which half maximum was reached by 13 h indicating an increased rate of use of the C1 and C2 fractions under non limiting O_2 conditions. In addition what was considered to be recalcitrant C1 and C2 fractions under the lower temperature is available under the higher temperature treatment. The difference between treatments 3/25 and 21/25 in the hour of half maximum is 34 h; that of 3/40 and 21/40 is 61 h. Based on these date is it possible that under different conditions the microbial populations are able to made use of the various carbon fractions at different points in time.

There are substantial changes occurring specific to the exit volatile gases and bacterial population under the four treatment conditions. Of all the ions detected by the GC/MS five were selected for the changes associated with treatment effect. The ions were tentatively associated with volatile compounds and detection was substantial under high temperature independent of O_2 levels, low O_2 conditions independent of temperature, independent of either O_2 or temperature, or dependent upon both elevated temperature and O_2. Based on the availability of the amendment (C1, C2, and C3), the hour of half maximum, and the total potential maximum CO_2 released, characteristic traits of the volatile compound source can be made. Compound A is the byproduct of

the microbial populations' ability to metabolize a product under both low and high O_2 conditions at the higher temperature setting (Figure 3.6). The heat increased the available fraction throughout the two treatments as confirmed under treatment 3/40 where compound A was only released once the target temperature was reached in treatment 3/40. Although produced under low O_2 conditions there is a sharp increase as the O_2 concentration increases after the hour of half maximum CO_2 which may be an indication of the ability to metabolize a recalcitrant fraction of the amendment and/or the microbial product. Compound B may be the byproduct of the microbial populations' ability to metabolize a product under both the low and high temperature setting at low O_2 conditions (3.7). The source metabolized by the microbial population releasing compound B, is of the C1 carbon fraction and necessitates either other precursor compounds to be had under the low O_2 conditions. Evidence in support of this conclusion is that compound B under both treatments peaked early on and is no longer detected well before the hour of half maximum CO_2, where C1 and C2 are no longer available. The differences in temperature did not increase the availability of the C1 fraction indicating that it is simple in its chemical composition. The microbial populations are active under anaerobic conditions.

Compound C was detected at hour zero at low levels which may indicate a low level release background from the amendment and/or the compost used (Figure 3.8). In all four treatments it was substantially detected which may be an indication that it is independent of the treatment settings. This compound is released under different O_2 and

temperature settings, and during different phases of available C1, C2, and C3 fractions, supporting a conclusion that the microbial carbon source is complex. Notable is the additional increase towards the end of treatment 3/40. The release of compound D is similar to that of compound B with a difference that the compound was detected at low levels at hour zero and 14 h which may indicate a background level coming from the amendment (Figure 3.9).

Compound E is released up to the hour of half maximum CO_2 primarily under the treatment of 21/40 and detected in treatments 3/40 and 21/25. The microbial populations are active under elevated temperatures. Under treatment 3/40 the compound is detected in conjunction with reaching the peak target temperature and continues to be released at low levels well beyond the hour of half maximum CO_2 which may be an indication that this microbial population is able to make use of recalcitrant forms of carbon or the microbial biomass. It is reasonable to consider the compound being produced by two different microbial populations, neither of which is able to undergo active growth under the conditions of reduced oxygen at the lower temperature setting (Figure 3.10).

Based on genetic materials (T-RFLP) there are bacterial population shifts occurring over time in the two sampled treatments 3% and 21% O_2 at 40 °C. There are 46 fragment lengths that were identified in treatment 3/40 at least once from the samples taken at P0, P1, P2, and P3 (T0, 27, 99, and 195 h). The identified fragments are detected at the same time with the tentatively identified compounds A-E (Figure 3.6-

3.10). A fragment length identified (the bacteria) as present at T0 may not be actively producing volatiles so it will not be exclude.

Compound A was not detected at T0, very low levels at 27 h, and clearly present at 99 and 195 h. Fragment lengths are present at the time are 55.22, 56.90, 152.53, 235.34, and 372.44. Compound B was not detected at T0, high levels at 27 h, and not detected at 99 and 195 h. The fragment length 240.36 is the only one out of 46 that fits the pattern.

Compound C was detected with low levels at T0, high levels at 27 h, marginally detected at 99, and at low levels at 195h. Fragment lengths present at the time are 224.59, 236.36, 578.90, and 580.64. Compound D detected at T0, high levels at 27 h, not detected at 99 and 195 h. There are no fragment lengths that fit the pattern. Compound E was not detected at T0, moderate levels just after 27 h, detected at very low levels at 99, and not detected at 195h. Fragment lengths that present are 239.03 and 240.36.

The bacterial population of the treatment with high O_2 and elevated temperature (21/40) was characterized using T-RFLP (Figure 3.13) as 62 fragment lengths identified at least once from the samples taken at P0, P2, and P3 (T0, 36.67, and 70 h) from the amended media (note: no sample evaluated at P1).

A heterotrophic population of mature compost had undergone a process of composting and diversity is maintained throughout as there is no dominate carbon source, changing O_2%, and temperature. The microbial population can be grouped as either r-strategists (high rates of reproduction) or K-strategists (optimal utilization of environmental resources) and in mature compost either dormant or active. The

amendment (Bradfield alfalfa fertilizer) used, is a complex carbon source that r and K-strategists are able to make use of at different rates during exponential, hump, and tail phases of the CO_2 gradient (%) leading to a succession of microbial populations over time. Characterization of the bacterial population T-RFLP under the treatment with low O_2 and elevated temperature (3/40) can be used to illustrate the succession. Of the 46 fragment lengths (bases) identified (Figure 3.11), the 26 selected as prominent, were enumerated and characterized 20 transient, 1 persistent-stable, and 5 persistent-non-stable (Figure 3.12). From the gas concentration (%), gradient (%), and respiration (g) of O_2 and CO_2, and hour at which the target temperature was reached (Figure 3.2) the conditions under which the fragment length was not detected or detected is known. From each group a few fragment lengths are selected to illustrate the dynamic population successions and an interpretative association to either the r or K-strategist.

The transient fragment length 574.36 was not able to establish a presence within the total population as of T0 through to P1 (26.83 h) under the increasing temperature and decreasing O_2 concentration. The detection at P2 with a height of 1748, occurred after the exponential and hump phases of CO_2 with O_2 concentration of 1.2% shortly after the hour of half maximum (89 h) CO_2 released at 40 °C. It was not detected at P3. These conditions could be an indication that the bacterium associated with the fragment length is an r-strategist with a high rate of reproduction given the correct conditions. Fragment length 240.36 was not detected at T0, but under increasing temperature, reduction of O_2 to 0.6%, well before (26.83 h) the hour of half maximum (89 h) CO_2

released, it was detected at P1 with a height of 206. As it was not detected in the two samples that followed the bacterium associated with the fragment length is a r-strategist able to have a high rate of reproduction from the available C1 and C2 fractions of the amendment. Fragment length 239.87 remained non detected though the changes of temperature and gas concentration, and the reduction of available C1 and C2 before the hour of maximum CO_2 released and was detected at P3 with a height of 357. These conditions would indicate that the bacterium associated with the fragment length is unable to establish detection under conditions dominated by others and it could be speculated that it made use of the microbial products as a source of energy for the high rate of reproduction. Fragment length 204.18 was present at T0 with a height of 664. It is no longer detected as the O_2 decreased and temperature increased to P1. At P2 is was with a height of 1420 which is more than a two fold increase at T0 after the exponential and hump phases of CO_2, with O_2 concentration of 1.2%, shortly after the hour of half maximum (89 h) CO_2 released, at 40 °C. It was not detected at P3. As this bacterium was able to have a high rate of reproduction given the correct conditions, and fluctuate between detected and non detected in successive samples, it is suggested that it belongs to the r-strategist group.

The only fragment length of the persistent-stable group is 56.90. With no change from T0 to P1 with a height of 467 and 469, with and increase height at P2 and P3 of 540 and 688 it is suggested that relative to the preceding fragment that this belongs to the K-strategist.

The phylogenetic changes of bacterial population was determined by the T-RFLP data (enzymes Hha I or Msp I) analyzed by inference in phylogenetic trees. The population detected using Hha I, evolving from least to greatest in similarity to the starting amended medium (P0), have a strong correlation to the accumulative respiration of CO_2 within the treatments (3/40 and 21/40) corresponding to the sample phase (P1, P2, P3). The population detected using Msp I, exhibits the same trend as that of Hha I with the exception of 3/40 P3 which is equal with least overall similarity as 3/40 P1. The population of 3/40 transitions three times between two groupings returning at P3 to be once again with least similarity with that of P0 despite the occurrence of greater accumulative CO_2 respiration.

Although the compost processes and resulting product offers a complexity of challenges in developing specific protocols, temperature, O_2% and moisture content can be economically managed in a bioreactor with a solid state amended medium. The system and concept are ready for further evaluation and production of activated compost water extract that can be tested for activity against specific plant pathogens.

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Appendix A

Construction of the Solid State Bioreactor as developed by Michael-Salomon Jost

Introduction

For the production of an Amended Compost Water Extract standard hardware such as a heat/refrigeration exchange unit, flow board, regulators are used in conjunction with a bioreactor developed by the author. A soup Thermos™ jug (internal volume of 0.5 L) is the structural base of the bioreactor. The first units developed, used aluminum foil as a lid and a thermometer to track internal temperature changes in a study of nematode population dynamics in amended mature compost. In the following section the "final" bioreactor (Figure 2.1.) is descried with possible modes of control or measurement after a general description of the support standard hardware used.

Materials and methods

Standard hardware

Regulation of internal temperature within the medium was done indirectly by either a vacuum glass insulator or a circulating water bath maintained by a heat/refrigeration unit. Within the bioreactor gas flow through the medium was regulated by a flow board distributing the gas at a constant rate to a multitude of ports. Before the gas went the distribution system it was sent through a scrubber and regulators and when needed a mixing chamber.

Bioreactor

The bioreactor developed has two parts that are temporally cemented together. A modified Thermos™ jug serves as the base and a lid constructed from foam board.

Modified Thermos Jug

Control or modification of temperature by addition or extraction of heat is achieved by circulating temperature regulated water around the structural unit holding the compost medium. In order to accomplish this, the following modifications were made to the Thermos™ jug. The bottom and the glass vacuum insulator are removed. Holes are drilled through the external housing 2 cm below and above the upper and lower rim respectively. Using a tool and die set, threads are tapped in both holes and a pair of polyethylene quick-disconnects. Prior to installing the quick-disconnects the treaded ends are cut short to 0.7 cm. Application of plumbers' silicone tape creates a seal of the threads as the quick-disconnects are mounted. The bottom of the jug is screwed back in place after applying two rounds of plumbers' silicone tape to the threads. The bottom and top ports are respectively where the in and outflow of the circulating water are attached.

If control of temperature is not a research objective then no modifications are made. The insulating properties of the glass vacuum surrounding the internal volume serves as a model of a compost pile mass.

Constructed lid

The lid is to provide increased insulation capacities beyond that of the original and a platform where tools of measurement or control can be fixed upon, or through to the medium within the bioreactor. In the following description the part of the lid that will be in contact with the internal part of the bioreactor will be referred to as lid bottom side; external lid top side. The sequence in which holes are drilled identifying numbers (i.e. #1) are designated.

Using a router with a strait bit and a jig, a 1.3 cm wide circle channel is cut out of the construction grade insulation fiber board (Owens Corning 150, width 5.1cm, insulation R value - 10) centered on a 3.8 cm radius. It is to fit snugly over the inner and outer edge of the jug opening. Using a jig, cut through the entire width leaving 2 cm of additional material outside the just cut channel. On the lid bottom side a 1.6 cm hole, 2.5 cm deep, is centered halfway between the radius center and the inner edge of the channel. Centered on the previous cut a 0.4 cm hole (#1) is made through the remaining material. Three addition holes (0.4 cm) are made through the foam board. Dead center (#2) and one each side of center (#3 & 4), 1/3 of the way between center and the channel, in relation to #1 and #2, so that angles 123 and 124 are 120°. On the lid top side a 2.8 cm keyhole bit is used to cut a 0.5 cm deep slot with the hole #1 slightly off center. In the hole #1 a 4 cm section of a Pyrex disposable 1 mL glass serological pipet (Corning company) pipette is inserted 3/4 of way and sealed in place using silicon caulk. When the dew point of the exit gas is higher than that of the external ambient, condensate will form on the inner walls and pool on the lid top. The height of the pipette segment will prevent condensate flowing back into bioreactor. In the channel made by the keyhole bit, a modified glass bottle (Corning) is inserted. The bottom has been cut off and a glass pipe (2.5 x 0.6 cm) attached at a right angle 1.5 cm below the top of the shoulder to which is fitted with a latex tube (5 x 0.5 cm) with a series of reduction segments ending with a silicon tube (0.2 x cm) with an overall length of 20 cm. The modified bottle is capped either with a rubber stopper, the manufacture's cap, or a SPME valve cap. The gasses exiting the bioreactor flow through the pipette and modified bottle. The SPME

valve cap is used to evaluate the exit gasses using appropriate protocol. By inserting a syringe in the latex tubing a sample can be taken directly from the exit gas flow.

The pipette inserted into hole #2 has the following additions are made. To its top end a glass T pipe is perpendicularly attached using a 1.5 cm section of silicon tubing. Attached to one of the arms of the T is a latex rubber tube of 20cm. Drawn through the tube and the length of the pipette a prepared ribbon of tissue paper (1 x 75 cm) is put in place using a fine wire so that the end is 2 cm from the pipette tip. The 1 cm wide paper is prepared into a paper cord, thereby reducing its width and increasing the tensile strength. This is done by attaching one end of the paper ribbon with tape to a bit in a drill. By running the drill and holding the end of the paper the cord is made. An extremely tight wrap is undesirable for the function that it is to perform. The objective is to with minimum numbers of revolutions, significantly increase the tensile strength and reduce the width, all the while maintaining the surface aria. A Day Pinchcock clamp has a loop which the latex tubing is brought through and then the free end is secured by the clamp. The clamp is then pushed until it rests at the junction of the glass T. The remaining free arm of the T is where the tube from the flow board will be attached once the base and lid are in place. The pipette and attachment is inserted through the lid in the hole #2 with the tip of the pipette 1.0 cm from the bottom of the internal lining of the bioreactor. During the joining of the two halves a separate source of positive gas flow is attached to the pipette so that, as it is lowered through the medium, particles are prevented from clogging the orifice. Once in place it is exchanged for the gas source coming from the flow board. For studies that need to have an elevated relative humidity that is self adjusting in relation to the temperature within the bioreactor, RO water is

injected with a syringe into the latex tubing attached to the T piece. As the water level increases it is forced up to the junction of the T and a few drops of water permitted to fall/flow down along the tissue paper within the pipette. The water level is retracted to where the latex tubing and the glass T meet. Water in the tubing is a reservoir, replacing water that is whisked away by the passing gas.

The pipettes to be placed in hole #3 and 4 have each a thermocouple wire pushed through until protruding 0.25 to 0.5 cm beyond the pipette. The pipettes respectively are pushed through the lid so that from the lid bottom they are 2 and 12 cm. If monitoring is not to be done then the thermocouples are removed and the ends of the pipettes topped off with a bead of silicon calk.

To take gas samples directly from a local within the medium two modified spinal tap needles (Monoject; gage and length; 18 x 6 inch) act as ports. The internal lance is removed and a head space is created by filling the top half of the needle head with silicon caulk. A syringe is used to draw samples from the head space. The units are installed between holes #3 & 4. To ensure a secure and air tight fit they are pushed through a bead of silicon dried on a 4 cm section of electrician tape mounted on the designated lid top side. They are pushed through the lid with only the tips emerging and only after the placement of the lid upon the bioreactor base are they pushed through into the substrate to a depth of 4 and 10 cm. In order to prevent particles of the substrate from clogging the open end as it is placed in position, a positive flow of gas is maintained by a decompressing a syringe that is inserted into the head space.

Union of the bioreactor lid and base

Prior to joining the two halves of the bioreactor a gasket is put in place to ensure that the flow of gas is directed through the exit port located at hole #1. Denture cream from a squeeze tube is applied on the entire top lip of the jug as a bead approximately 0.5 x 0.25 cm. As the lid is brought into place, judgment is needed as the cream needs only to touch both the two joining segments to provide the desired function.

The "final model" as presented is by no means the end all. Further reflection upon simple innovations will expand the capacities of this device.

Appendix B

Preliminary Production and Testing of Aerated Compost Tea

Introduction

A five-acre orchard with over 2500 trees was planted at the MSU Clarksville Horticulture Research Station (CHES) during the 2000 growing season. The primary objectives of the project include a) the characterization of soil quality changes with transition to organic management practices, b) a study of the interaction of ground floor management strategies with apple root stock vigor, c) studies of the marketing potential and considerations for organic apples, d) educational programming and materials related to organic orchard management, and e) management of biological diversity for control of fruit and foliar pests and pathogens.

Standards regulating organic farming practices limit the use of products that be used as controls for pathogens. In the mid-west United States, apple scab (*Venturia inaequalis*) is an economically important foliar pathogen. The most common organic management tool is the application of foliar sprays of sulfur or lime sulfur (Bevan and Knight, 2001; Edwards, 1998; Phillips, 1998; Swezey, 2000). Yohalem et al. (1994) reported the use of a fermented anaerobic compost water extract (also known as anaerobic compost tea) as having potential as a viable form of biocontrol on conidia of diseases on maize, apple, red pine, and ginseng. The compost used was spent media from commercial button mushroom production. Under laboratory conditions in vitro the product used to challenge the spore suspension ion was reported to significantly inhibit germination. The most effective extracts were obtained between five and nine days of non aerated fermentation. The product was also effective after being autoclaved or filter sterilized. The development of disease was inhibited on the seedlings maize, apple, red pine, but not ginseng, grown in growth chambers. Results reported in by Yohalem et al.

(1996) from the field trial are of mixed success using the same protocol for production of the compost derived water extract, in a later study over three consecutive growing seasons, apple trees in two different orchards susceptible to apple scab were evaluated for the treatment effect of the foliar application. The extracts significantly reduced both the leaf area affected and the disease incidence better than the negative water control but not as well as the conventional chemical compound Captan. The first year the total numbers of microorganisms on the leaf surface to be greater on that receiving the compost derived product in contrast to that receiving the conventional fungicide Captan. The following year the total number of organisms was similar with the respective product treatment but microbial diversity was significantly different.

The production of aerated brewed compost tea is a process that involves the mixing and fermentation of compost, water, and any optional amendments in a "compost tea brewer". Compost used is of a good quality, stabilized and is free of phytotoxic compounds. Water is used as the medium for the extraction and fermentation of microbes and abiotic compounds. Some production protocols call for the addition of amendments ranging from sugar to rock dust as an energy source for the exponential growth of the microbial population. The brewing process is to take place in a device where aerobic conditions and temperatures near 22 °C are maintained. There is an abundance of designs described by the National Sustainable Agriculture Information Service (ATTRA) (http://attra.ncat.org/) which can be categorized into two groups. The first are homemade made from readily available low-cost materials, with simple construction and maintenance. The second are commercially manufactured by individuals or organizations making claims that the device is capable of producing

aerated brewed compost tea. As indicative of the product's name, it is an aerated aerobic process achieved by incorporating atmospheric air and maintaining minimum saturated O_2 levels. Some of the devices have a mixing/agitating device to keep the fluid in flux. The recommended brewing cycle is one to two days at which point the tea is removed and minimally filtered before application.

The primary objective of the research reported here was to develop a protocol for production and application of aerated brewed compost tea at the CHES organic apple research plot and to test the efficacy on apple scab. Greenhouse grown cucumber seedlings were used as a bioassay for systemic acquired resistance. The product was also provided for testing by other researchers on grape, raspberry, and strawberry for management of foliar and fruit disease under field and post harvest conditions.

General Materials and Methods

Compost

Compost was made specifically to be used in the production of aerated brewed compost tea. As the volume of compost needed is small relative to other compost applications, it is deemed reasonable to produce "designer compost". Designer compost is based on a conceptual idea that, although incurring higher costs per cubic meter, the product can be reproduced. Using readily available materials six different composts were made based on volume/volume mix ratio. The dairy manure used was from a tie stall and had a small percentage of sawdust that came from the bedding. The swine manure slurry used had some of the liquids extracted so that the moisture content was 75-80%. The

leaves used were gathered in the fall from deciduous broad leaf trees and stored out doors in a pile for six moths before use. The field corn was harvested using a chopper as would be for silage where the entire aerial structure is reduced to chips approximately 10 X 4 mm.

The first compost (#1) was made with a material mix of 20% dairy manure, 20% swine manure, 15% each alfalfa hay, grass hay, leaves, chopped corn, and six pounds of sulfur per cubic yard. The second compost (#2) mix was 36% sphagnum peat, 16% alfalfa hay, 16% grass hay, 16% leaves, and 16% chopped corn. The third compost (#3) was made with a material mix of 20% dairy manure, 20% swine manure, and 15% each alfalfa hay, grass hay, leaves, chopped corn. The forth compost (#4) was made from 40% swine manure mixed with 60% wood shavings. The wood shavings were 2-3 mm in size made from soft wood and are typically used in pavilion show horse stalls. The fifth compost (#5 (Dairy)) mix was made from 50% dairy manure (tie stall/bedding-wood shavings), 25% straw, and 25% grass hay. The sixth (#6 (D)) was made with a material mix of 20% dairy manure, 20 % swine manure, 20% sphagnum peat, 10% alfalfa hay, 10% grass hay, 10% leaves, and 10% chopped corn.

The materials of the fourth and fifth compost were layered in their respective windrow and mixed using an overhead self propelled compost turner. The preceding composts were obtained by alternately layering the materials in a manure spreader (4-6 cubic yards) before running the materials through the spreader to create a windrow pile. All the windrows were turned on a weekly basis for at least five consecutive weeks with an overhead self-propelled compost turner. The composts were made eight to ten months

before being used. From each compost pile, half a cubic meter was placed in a wood storage bin for later use in the production of aerated brewed compost tea.

Brewer

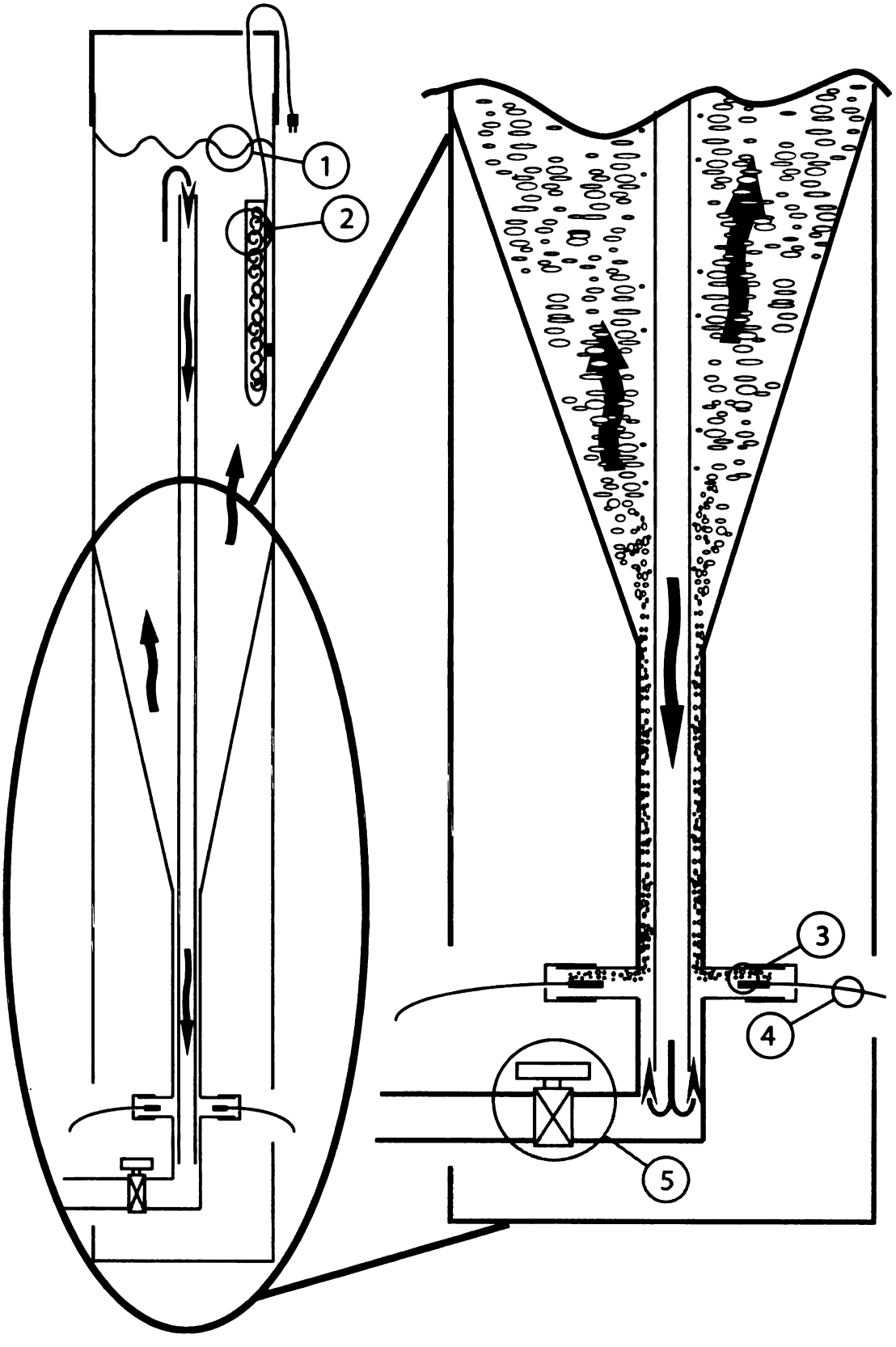
Throughout the previous growing season (2001) a small scale exploratory project was implemented in the production and application of a compost tea for the control of apple scab. The water extraction or fermentation was done for 48-72 hours in a battery of 2.5 gallon buckets with and without aeration as provided by an aquarium pump and aeration stone. Towards the end of the season 55 gallon barrels were used. The tea product was made of various ratios of water and compost or plant materials. Some of the mixtures received an amendment of sugar as a readily available energy source. After sieving the product it was applied with a designated spray rig on the orchard. Invaluable experience was gained in regards to the "how to" and "know how" for the large scale production and application for the following year. Although not successful, an attempt was made to quantify the number of colony forming units over time from samples taken from the buckets on a potato dextrose media.

In contrast to the production methods as used by Yohalem et al. (1994) and others by anaerobic fermentation, a method known as aerated brewed compost tea has recently gained attention in the USA. The methods were obtained at a seminar at an Acres USA® conference as outlined by E. Ingham (Ingham, 2003; Ingham and Anderson, 2003). Addressing a primary concern with this production method of anaerobic hot spots within the production cycle the 2.5 and 55 gallon vessels were abandoned. For the 2002 season a bioreactor was designed and developed for the production of product for the organic apple orchard of MSU.

A review of the "compost tea brewers" commercially produced for the end user market and publicly available published home made designs, revealed that the commercial units were too expensive and the home made designs were unsatisfactory. Having identified the short comings, a team of students at MSU set out to develop a brewer/bioreactor with other attributes. One of the goals was that if the research provided applicable results, the means of acquiring the bioreactor needed to be accessible to a wide range of researchers and farmers. It was to be built from readily available off the shelf components and be easy to construct. The second goal was that the compost was to remain suspended in the water, in contrast to having it in a soaker bag, so as not to create hot spots where oxygen depletion could occur over an extend period. The last factor was that the devices needed to be large enough for field scale research use but not so large that it would not be feasible to install in a research laboratory. If a larger number of units were needed for either total volume or replicates, a battery of the bioreactors could be maintained.

Six 20 liter compost tea brewers were built. The materials needed for the constructing and operation of the brewer are PVC piping, fittings (end caps, valves, y-branches, etc.), and adhesive, silicone caulk, Teflon tubing, fish tank heater and spurges, and a source of clean compressed air. The most difficult section of construction was the formation of the internal cone. Using a template the material was cut from the large PVC pipe. The material was placed in hot water steamer to make it malleable in order to shape it. For the conceptual design see Figure Appendix B.1.

Figure Appendix B.1. Conceptual design of construction for a 20 liter compost tea brewer with key: 1) Water line, 2) Heater, 3) Air sparge, 4) Compressed air in flow hose, and 5) Drain


1
2
3
4
5

Aerated brewed compost tea – production

The brewer was filled with 20 liters of RO water and allowed to equilibrate to the desired temperature for one hour while compressed air was injected into the system. The compost, previously described, was screened through a 0.5 x 0.5 cm hardware cloth to remove any large particles prior to adding it to the water at a volumetric ratio of 1:10. The rising air bubbles create an updraft providing for continual circulation of the solution. Due to the evaporation of water from the brewers the water levels were maintained by the addition of RO water once a day. At the time, a decision was made not to add any amendment to the brewing process. Once the brewer was in production mode a simple aquarium heater was used to mitigate the diurnal temperature fluctuations of the production location and the resulting evaporative cooling by the introduction of compressed atmospheric air.

The duration of the brew cycle was often dictated by last minute changes in the spray schedule. The wide time frame of production varied between 12 and 120 hours. The contents at the time of removal from the brewer were filtered through a double ply mosquito screen as it flowed to the containers for transport. The solids were discarded and the remaining liquid was the aerated brewed compost tea used in the studies.

Specific Methods and Results

Three methods were used to test the activity of the extracts.

1. Field application to apple trees at CHES organic apple plot for management of apple scab

2. Application to greenhouse grown cucumber seedlings using an established protocol for testing the potential to elicit a systemic acquired resistance (SAR) response
3. Field testing with synthetic chemical control agent for activity against foliar and fruit pathogens of grape, strawberry and raspberry. Specific protocols used are presented with results for each method.

CHES Organic Apples

Location/materials/methods

An evaluation for the fungicidal properties of various sprays was conducted for management of apple scab (*Venturia inaequalis*) in apple (*Malus pumila* Rosaceae, 'Buckeye Gala/M.9, Goldrush/M.9, Smoothee Golden Delicious / G.16') at the CHES Organic Apple orchard. The orchard was planted in a random complete block design. For each of the three varieties 100 trees made up each block, with three blocks per section. Two sections were used for this experiment. Each section was divided in half to allow for four treatments. A single row of trees on each side of the treatments was used as a buffer. Cultural management of the high density planted orchard spaced at 4 x 15 ft was hand pruning, central axis trained to trellis. The orchard received treatments throughout the season of sulfur/lime-sulfur, aerated brewed compost tea, Seranade™, and water as a negative control. The application of the sprays was done using a Proptec™ Rotary Atomizers tractor pulled-styled prototype sprayer that delivered the product at low rates at a pressure near that of ambient. Sulfur or lime-sulfur was applied at a rate of 1 gallon per acre eight times. Aerated brewed compost tea was made using compost #6 (previously described) (extraction 45-50 hours) and applied five times at a rate of 5

gallons per acre. Seranade was applied at a rate of 12 ounces product per acre six times. Seasonal and weather factors played an important part in determining dates so as to meet target phonological stages of plant/pathogen development. The evaluation of scab incidence by late summer was done by harvesting 20 randomly selected shoots per variety, per replicate, and counting the number of leaves out of eight that were infected with scab lesions.

Results and Discussion

The cultivar Goldrush/M.9 is resistant and subsequently there was no infection for all treatments. The presence of the vector of apple scab for the growing season was evidenced by the infection on leaves of susceptible apple varieties under the treatment of water as the negative control. The average of number of leaves infected per shoot of eight fully expanded leaves reported for the lime sulfur, aerated brewed compost tea, Seranade and a negative control were 0.83, 3.0, 2.08, 2.40 and 0.65, 3.65, 1.65, 3.13 for the cultivars Buckeye Gala and Smoothee Golden Delicious. Dividing the two blocks of trees into four large nonreplicated segments forced an assumption to be used for the statistical analysis using ANOVA. The results therefore can only be used as very strong trend indicators. While there are significant differences between the negative control and the lime sulfur, there is none between the aerated brewed compost tea, Seranade and negative control.

Greenhouse Cucumber - seedling bioassay for SAR

Location/materials/methods

Cucumber plants (*Cucumis sativus* L. cv. Wisconsin SMR 58) were used as a bioassay to investigate if the application of aerated brewed compost tea induced a response of an systemic acquired resistance (Hammerschmidt, 1982). Individual cucumber plants were established in plastic pots with a soilless potting media (Pro-Mix BX). Once the first leaves were fully expanded, sixty four plants were selected for uniformity and randomly placed upon the green house bench. Groups of eight plants were used for each of the treatments. The treatment spray was applied to the expanded leaf (emerging second leaf was covered) on both sides until dripping wet using a "cleaning spray bottle hand pump". The spray treatments were RO water and Actiguard® (20 ppm a.i.) as respective negative and positive control, and six different aerated brewed compost teas from the previously descried composts #1-6. The duration of the extraction was 58 hours without amendment. The plants were grown until the second leaf of all treatments was fully expanded at which point the second leaf was harvested. In accordance with the post harvest protocol the leaves were cut in half lengthwise and one of the halves individually placed in a covered Petri dish lined with a moistened filter paper. Droplets of a spore suspension of pathogen Anthracnose *(Colletotrichum lagenarium)* was placed on ten sights on the leaf and incubated at ambient temperature. When the disease had advanced sufficiently on the control all treatments were evaluated for both the number and size of the lesions.

Results and Discussion

The average of number of developed infection sites per inoculated leaf for the negative control, aerated brewed compost teas (#1-6), and positive control is 9.4, 9.8, 9.6, 8.0, 7.1, 9.3, 4.3, and 0.9 respectively (Figure Appendix B.1). The average size

(mm^2) of the developed infection sites of the same treatments are respectively 14.9, 26.7, 24.3, 17.0, 21.9, 12.3, 9.2, and 17.6. (Figure Appendix B.2)

Figure Appendix B.2. Average number of lesions of ten inoculated Anthracnose (*Colletotrichum lagenarium*)of incubated cucumber leaves (*Cucumis sativus* L. cv. Wisconsin SMR 58) after the application of pretreatment sprays.

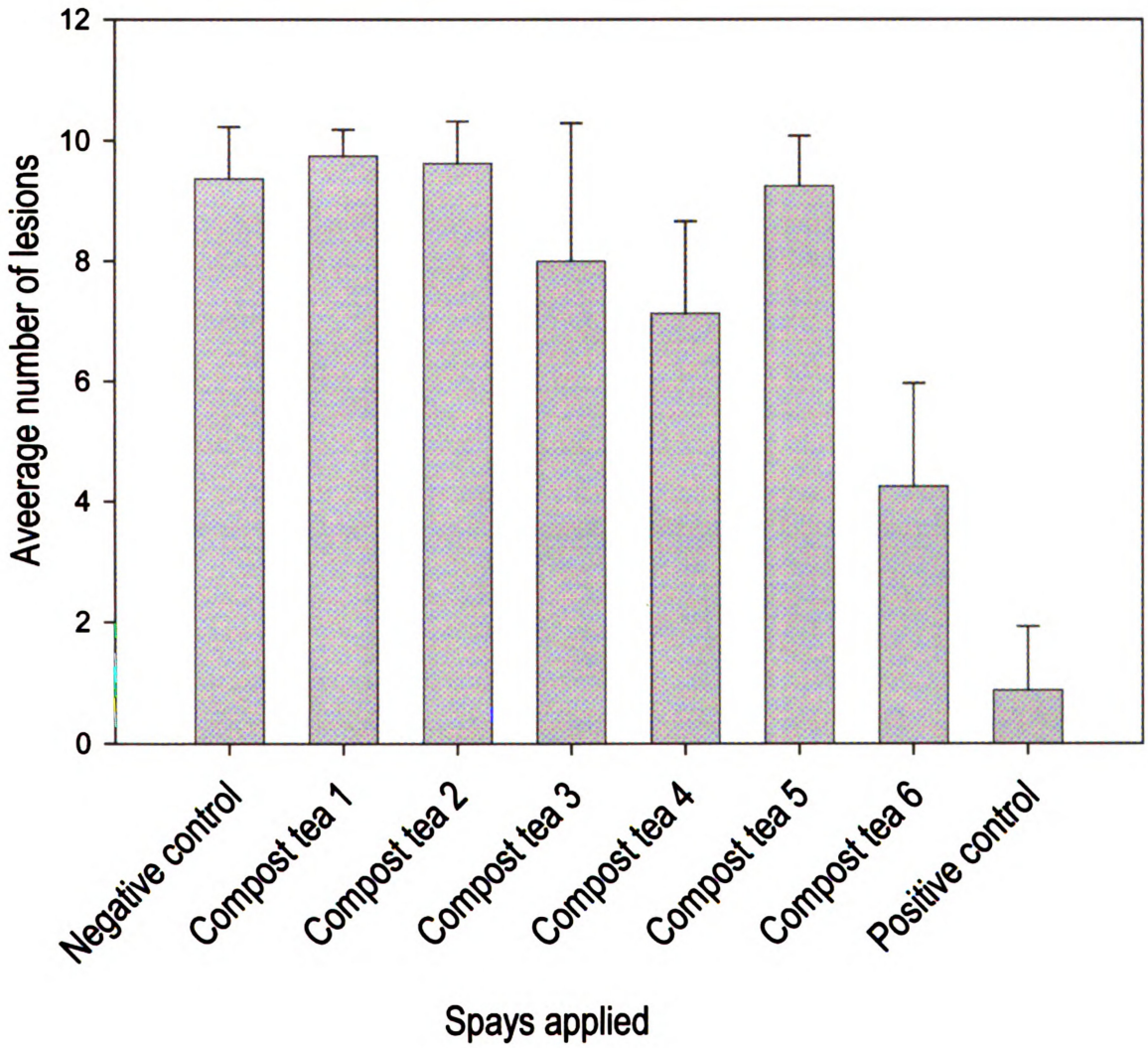
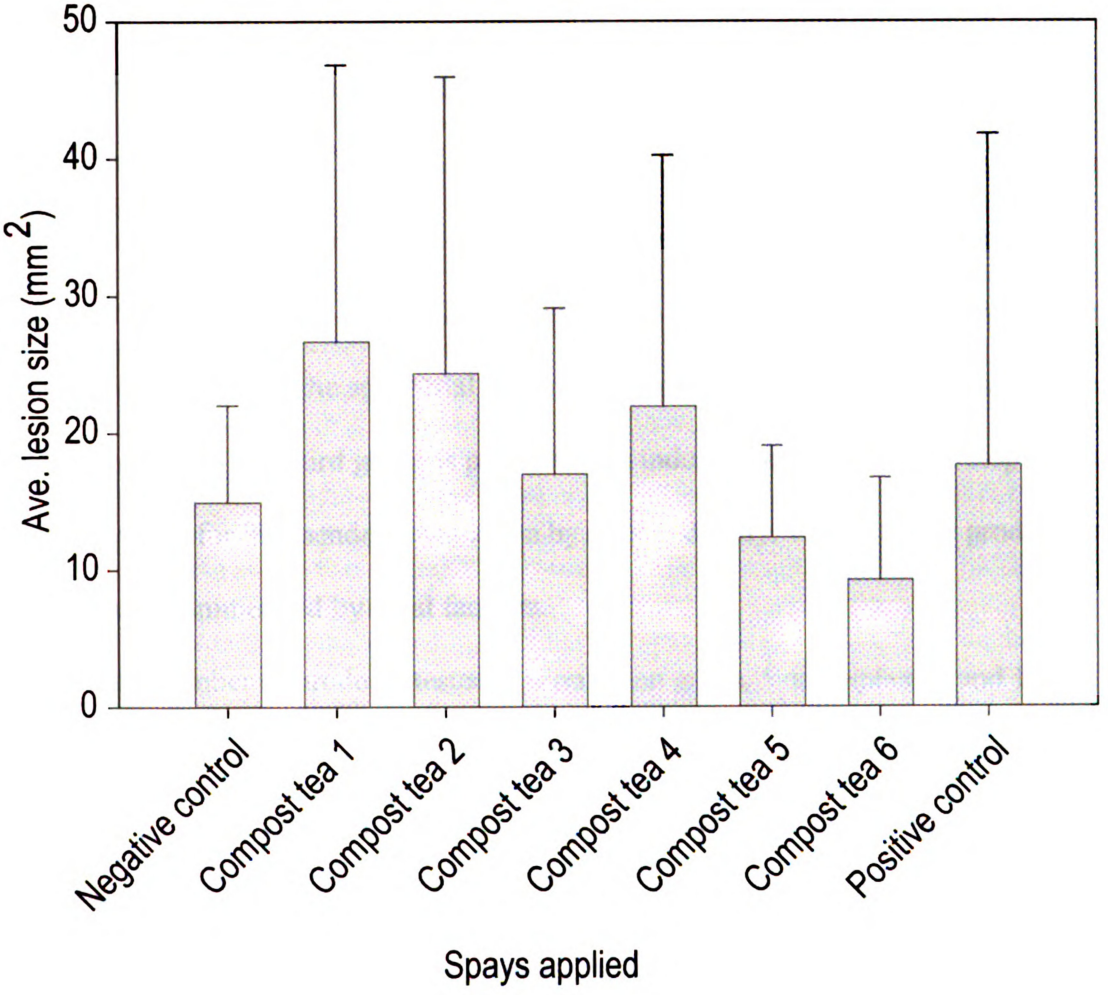

12
10
8
6
4
2
0
Aveerage number of lesions
Negative control
Compost tea 1
Compost tea 2
Compost tea 3
Compost tea 4
Compost tea 5
Compost tea 6
Positive control
Spays applied

Figure Appendix B.3. Average lesion size of inoculated Anthracnose (*Colletotrichum lagenarium*) of incubated cucumber leaves (*Cucumis sativus* L. cv. Wisconsin SMR 58) after the application of pretreatment sprays.


50
40
30
20
10
0
Ave. lesion size (mm 2)
Negative control
Compost tea 1
Compost tea 2
Compost tea 3
Compost tea 4
Compost tea 5
Compost tea 6
Positive control
Spays applied

One of the six aerated brewed compost teas did reduce the number of lesions by one half (Figure Appendix B.1). There are no differences as a result of the treatment application of the average lesion size once the disease established itself within the plant tissue (Figure Appendix B.2) Based on these results further investigation to determine the levels of systemic peroxidase activity within the leaf tissue was not pursued.

Spray trials for foliar and fruit pathogens of grape, raspberry, and strawberry

The products used in the spray trials can be characterized in general terms. The first group is used in controlling disease on specific commercially grown crops and is referred to as the industry standard. The second was the use of an industry standard on "non labeled crops" in the spray trial as part of the process to receive approval for use on additional crops. The third group is product from industry research and development that were provided for independent evaluation by a second party. The last were products of interest as recommended by local farmers.

The number of products tested was on 10 on grape, 5 on raspberry, and 15 on strawberry. The test products range from chemical compounds, pure biological culture, and aerated brewed compost tea. In the spray trial the industry standard and water are the positive and negative control respectively. The test aerobic brewed compost tea (non amended) was made from compost # 2 (previously described) with an extraction time ranging from 12-120 hours.

Data acquired by the experiments was prepared for statistical analysis by establishing the values of means using either the arcsin-transformed data or log(x+1)-transformed data. To substantiate the differences of the treatments in relation to the untreated control statistical analysis was preformed using Fischer's Protected LSD test

(P≤0.05) or *(P≤0.10)*. The field experiments and evaluation results (all products and all plants) were not directly a part of this thesis project. As the aerated brewed compost tea and funding was provided for the field trials, pertinent information was extracted and reported in the following section. The full report is authored by Schilder et al. (2002).

Grape

Location/materials/methods

An evaluation for the fungicidal properties of various sprays was conducted for control of disease upon the host plant material. The subject of study are the foliar and fruit diseases Black rot (*Guignardia bidwellii)* and Downy mildew (*Plasmopara viticola*) in commercially grown grape (*Vitis labrusca* 'Niagara'). On the Trevor Nichols Research Complex in Fennville Michigan, a 3-yr-old vineyard was selected for the experiment. Between the treatments one-vine buffers were used. Cultural management of the plants was hand pruning, cordon trained, and spaced at 6 x 10 ft. Each treatment was replicate 4 times in a randomized complete block design using a plot made of 3-vines. The application of the sprays was done using a cart-styled research sprayer that delivered the product at a rate of 75 gallons per acre at a pressure of 55 psi. A total of up to five spray applications were made. As it was a field experiment seasonal and weather factors played an important part in determining dates so as to meet target phenological stages of plant development. For both diseases, the severity is reported as a calculated value based on (incidence x severity)/100. For downy mildew the value was based on 25 randomly selected leaves from the center vine of each plot which were visibly inspected to estimate both the incidence and severity of the infected leaves. Reported values are the means. Statistical analysis was preformed on log (x+1)-transformed data and

significance determined according to Fisher's Protected LSD test *(P≤0.05)*. For Black rot evaluation 10 randomly selected fruit clusters per plot were evaluated in the same fashion as described as above. Reported values are the means. Statistical analysis was preformed on arcsin-transformed data significance determined according to Fisher's Protected LSD test *(P≤0.10)*.

Results

The spray treatments applied for control of downy mildew (*Plasmopara viticola*) on the foliage of grape (*Vitis labrusca* 'Niagara') all significantly reduced the severity of the pathogen during a season of relatively low pressure. The reported treatments, as a percentage overall severity value, for untreated control, aerated brewed compost tea, and Dithane (Active ingredient: Mancozeb)/BAS 516 (Active ingredient: Pyraclostrobin (BAS 500) + boscalid (BAS 510)) is 16.7, 2.7, and 0.4 respectively. In regards to the disease black rot (*Guignardia bidwellii)* on fruit, the seasonal pressure was low. Using the same overall severity percentage the treatments of untreated control, aerated brewed compost tea, and BAS 516 had values of 5.23, 3.28, and 0.0 respectively. While there are significant differences between the untreated control and BAS 516, there are none between the aerated brewed compost tea and the untreated control and BAS 516.

Raspberry

Location/materials/methods

The subject of study are the cane and leaf diseases, and fruit rots, Spur blight *(Didymella applanata)*, Cane blight *(Leptosphaeria coniothyrium)*, Anthracnose *(Elsinoe veneta)*, Leaf spot *(Sphaerulina rubi)*, Cladosporium rot *(Cladosporium* spp.*)*, and Gray

mold, *(Botrytis cinerea)* in red raspberry *(Rubus idaeus* 'Tulameen'*)*. On a commercial red raspberry planting in South Lyon, Michigan, four replicated 10 ft row sections were set up in complete a randomized block design with a row spacing of 10 ft. The application of the sprays was done using a cart-styled research sprayer that delivered the product at a rate of 50 gallons per acre at a pressure of 40 psi. A total of up to five spray applications were made. As it was a field experiment seasonal and weather factors played an important part in determining dates so as to meet target phenological stages of plant development. Three weeks after the last spray healthy-looking berries were harvested and placed under post harvest conditions where after four days fruit with more than 10% area coved were counted and organisms identified. A month after the last spray application a visual estimate from the center 3 ft of each plot and 10 canes were evaluated for leaf spot severity, as a percentage of leaf area infected by causal agents anthracnose, and the more difficult to differentiate spur blight/cane blight. Calculating a percent value by the number of canes diseased of the total number of canes selected the disease incidence was established. The following spring from each of the plots the center 3 feet were evaluated for the number of live floricanes. Reported values are the means. Statistical analysis was preformed on log (x+1)-transformed data significance determined according to Fisher's Protected LSD test *($P \leq 0.05$)*.

Results

Results varied for the treatments applied to red raspberry *(Rubus idaeus* 'Tulameen'*)* for the diseases of Leaf spot *(Sphaerulina rubi)*, Anthracnose *(Elsinoe veneta)*, Spur blight *(Didymella applanata)*, Cane blight (Leptosphaeria coniothyrium), Cladosporium rot *(Cladosporium* spp.*)*, and Gray mold *(Botrytis cinerea)*. The foliar

disease pressure by leaf spot was low. All five treatments significantly altered the severity of the disease. Severity reduction for aerated brewed compost tea, Captan/Nova, and the control was 0.13, 0.15, and 4.50 respectively. Moderate pressure by the cane diseases, anthracnose, spurblight and caneblight was in part effectively reduced by some of the spray treatments. Anthracnose incidence was significantly reduced by all five treatments in comparison with an untreated control. Aerated brewed compost tea and two other products in the trial had values of 10.0 in comparison with 77.5 of the untreated control. Although differences are not significant, values of spurblight and caneblight incidence for aerated brewed compost tea, Captan/Nova and the untreated control were 12.5, 15.0, and 32.5 respectively. Fruit rot diseases botrytis and cladosporium rot, were to varying degrees controlled by the treatments. Aerated brewed compost tea did not test as providing significant differences between the untreated control and the Captan/Nova that did significantly reduce the indices to 43.0 from the comparison made at 64.5. Some of the treatments significantly increased the ability of the canes to survive through to the next growing season. In the three foot section selected the untreated control had 2.75 live canes, aerated brewed compost tea 7.25, and Captan/Abound 17.75. There was no significant difference between the control and aerated brewed compost tea.

Strawberry

Location/materials

An evaluation for the fungicidal properties of various sprays was conducted for control of disease upon the foliage, calyx, fruit, and post harvest fruit of the host plant. The site selected for the experiment was 4-yr-old, commercial, matted-row strawberry planting in Onondaga, Michigan. With no buffer rows, each treatment was replicated

four times in a randomized complete block design with rows spaced 42 inches apart. The treatment section was a 10 ft section of the row. The application of the sprays was done using a cart-styled research sprayer that delivered the product at a rate of 100 gallons per acre at a pressure of 55 psi. A total of five spray applications were made for each of the 15 products between the phenological stages from pre-bloom through to when the fruit was full ripe.

Strawberry: Foliage

Methods

The subjects of study are the foliar diseases Phomopsis leaf blight *(Phomopsis obscurans)* and Leaf spot *(Mycosphaerella fragariae)* of commercially grown strawberry (*Fragaria x ananassa* 'Redchief'). Approximately three and a half weeks after the last spray the leaves were evaluated for leaf blight and leaf spot. Severity was established by the visual estimation of the total leaf area and the percent area affected. Samples were taken from the center 3 ft of each plot. Reported are the mean values. Statistical analysis was done and significance determined according to Fisher's Protected LSD test *($P \leq 0.05$)*.

Results

The reduction of Phomopsis leaf blight *(Phomopsis obscurans)* severity under moderate pressure was significantly reduced by aerated brewed compost tea and Captan/Benlate/Kocide. The untreated control, aerated brewed compost tea, and Captan/Benlate/Kocide had percent affected leaf area of 12.8, 6.0, and 3.8 respectively. Leaf spot *(Mycosphaerella fragariae)* affected 2.0 percent of the untreated control with

disease pressure characterized as low. Aerated brewed compost tea was able to reduce the affected leaf area to 0.7 and so too was the BAS 516 to below 0.01 percent. The reductions are statistically significant.

Strawberry: Calyx

Methods

The subject of study are the calyx diseases Leaf scorch *(Diplocarpon earlianum)* and Angular leaf spot *(Xanthomonas fragariae)* of strawberry *(Fragaria x ananassa* 'Redchief'*)*. Thirteen and twenty days after the final spray marketable berries were harvested from the center 3 ft of each treatment row. Of marketable berries 25 were randomly selected for evaluation. If disease symptoms occupied more than 1/3 of the calyx the significance was rated. Reported values are the means. Data from angular leaf spot first and second harvest was transformed for statistical analysis using arcsin and log (x+1) respectively. Statistical analysis was preformed on the data from both diseases and significance determined according to Fisher's Protected LSD test *($P \leq 0.05$)*.

Results

The scorch *(Diplocarpon earlianum)* incidence on the calyx of the fruit of strawberry *(Fragaria x ananassa* 'Redchief'*)* at the first harvest by all treatments was not significant. In the second harvest the reduction by aerated brewed compost tea was significant when compared to the control, and that which provided the greatest reduction, BAS 516, with values of 21.0, 50.0, and 13.0 respectively.

Results from the application of various sprays in order to study their efficacy in reducing angular leaf spot *(Xanthomonas fragariae)* on the calyx during the first harvest

did not result in significant differences in relation to the untreated control. When compared to the untreated control during the second harvest there are statistical differences in the reduction of angular leaf spot. The respective values for the untreated control, *Paenibacillus macerans* cocktail with two other products, and aerated brewed compost tea are 9.0, 0.0, and 1.0. underlined.

Strawberry: Fruit

Methods

The subjects of study are the field rot diseases of Botrytis gray mold *(Botrytis cinerea)*, Tan-brown rot *(Hainesia lythri)*, Anthracnose fruit rot *(Colletotrichum* spp.*)* in strawberry (*Fragaria x ananassa* 'Redchief'*)*.Three days after the last spray all berries were harvested from the center 3 feet of the plots. Marketable fruit were incubated in clear containers for two days at 25 °C with ambient light. Fruit field rot was calculated in percentage format of the rotted fruit over total fruit. Reported are the means values. The data from botrytis gray mold and anthracnose fruit rot changed to log (x+1)-transformed for statistical analysis. All three diseases were evaluated to determine significance according to Fisher's Protected LSD test *($P \leq 0.05$)*.

Results

Overall incidence was low for three field rot diseases, Botrytis gray mold *(Botrytis cinerea)*, and Anthracnose fruit rot *(Colletotrichum* spp.*)*, Tan-brown rot *(Hainesia lythri)*, on fruit of strawberry (*Fragaria x ananassa* 'Redchief'*)*. Botrytis gray mold was controlled by elimination of any symptoms by the use of Captan+Benlate+Kocide. Aerated brewed compost tea did not significantly reduce the

pathogen with a value of 2.5 in comparison with the untreated control of 3.0. Results from the spray program for tan-brow rot were statistically significant but were deemed as inconclusive as a result of the patchy nature of the infection's distribution in the field. Anthracnose reduction by aerated brewed compost tea was not statistically different from the untreated control with values of 5.3 and 2.5 respectively. Captan+Benlate+Kocide did reduce any symptoms of the disease.

Strawberry: Fruit – post harvest

Methods

The diseases of study are the post harvest fruit rots, Tan-brown rot *(Hainesia lythri)*, Botrytis gray mold *(Botrytis cinerea)*, Rhizopus rot *(Rhizopus spp.),* Anthracnose fruit rot *(Colletotrichum* spp.*)* of commercially grown strawberry (*Fragaria x ananassa* 'Redchief'*)*. Three and ten days after the last spray all marketable berries were harvested from the center 3 feet of the plots. Berries were placed in an incubation chamber for 7 days thereafter visually assessed for signs of sporulation and identification. The evaluation was done on fruit from two different harvest dates. Reported values are the means. Data from anthracnose fruit rot and botrytis gray mold for statistical analysis was preformed on arcsin-transformed data and significance determined according to Fisher's Protected LSD test *($P \leq 0.05$)* for the first harvest and Fisher's Protected LSD test *($P \leq 0.10$)* for the second. The data for rhizopus rot and tan-brown rot was statistically analyzed to determine significance according to Fisher's Protected LSD test *($P \leq 0.05$)*.

Results

Anthracnose fruit rot at the first harvest was not statistically reduced by aerated brewed compost tea when compared to untreated control, though by the industry standard it was as incidence percentage of 52.0, 12.0, 2.0 respectively. The prevalence of disease was less in the second harvest. Untreated control had a value of 13.0, aerated brewed compost tea 13.0, and the industry standard 1.0. For Botrytis gray mold there were no statistical differences between any of the treatments at either harvest with the control values of 27.0 and 13.0. Rhizopus rot was not significantly reduced in the first harvest by any of the treatments. In the second harvest the aerated brewed compost tea percent incidence in relation to the untreated control was not significantly different with the respective values of 56.0 and 77.0. The use of Captan+Benlate+Kocide did reduce the value to 8.0 and in the case of the second was significantly different. The reduction by treatments of tan-brown rot was not statistically significant for either of the harvests.

Discussion

Spray test trials were conducted in the field and lab on grape, red raspberry, and strawberry evaluating the products upon their effect on disease and the respective vector upon the host tissue in either a field or lab setting. There are no statistical differences between the untreated control and any of the applied products in 7 of the 24 experiments. An explanation for one of the experiments is due to the extremely low disease incidence.

Of the 17 trials where statistical significances were detected between the untreated control and at least one of the test products, a few general statements can be made in regards to untreated control (UC), aerated brewed compost tea (CT), and industry standard product (ISP). No statistical difference was established between the ISP and CT in 12 of the experiments, 5 of which the CT treatment had a greater

reduction of disease indices. Disease reduction by the use of CT was significant in comparison with the UC under 8 of the 17 evaluations.

As the primary focus of this report is aerated brewed compost tea, the differences and in depth review was not done on the other products. The results pertaining to aerated brewed compost tea in relation to the industry standard and the untreated control are very encouraging.

The evaluations done on the three plants, disease and vector, location of testing, and the plant structure are summarized in Table Appendix A.1. The treatments selected for summary are the untreated control (UC), aerated brewed compost tea (CT), industry standard product (ISP), best research test product (BRTP). In each evaluation the treatments are ordered by the disease incidence (low to high) followed by the statistical significance of the product against all the products.

Table Appendix B.1. Summary performance of selected spray treatments (Untreated control (UC), Aerated brewed compost tea (CT), Industry standard product (ISP), Best research test product (BRTP)) in the reduction of disease in field trials of grape, raspberry, and strawberry.

Plant	Disease; *agent*, setting, structure	Reduction of disease by treatments (low to high)	Fisher's Protected LSD test *(P≤0.05)* or **(P≤0.10)*
GRAPE; *Vitis labrusca* 'Niagara'	Downy mildew; *Plasmopara viticola*, field, leaves	UC<ISP<CT<BRTP	a ; bc; bcd; d
	Black rot;*Guignardia bidwellii*, field, fruit	UC<CT<ISP<BRTP	*a; ab; ab; d
RED RASBERRY *Rubus idaeus* 'Tulameen'	Gray mold; *Botrytis cinerea* / Cladosporium rot; *Cladosporium* spp. lab field, fruit	UC<CT<BRTP<ISP	a; ab; b; b
	Leaf spot; *Sphaerulina rubi*, field, leaves	UC<BRTP<ISP<CT	a; c; c; c
	Anthracnose; *Elsinoe veneta*, field, cane	UC<ISP=BRTP=CT	a; b; b; b
	Spur blight; *Didymella applanata* / Cane blight; *Leptosphaeria coniothyrium* field, cane	UC<BRTP<ISP<CT	*ns*
	Live canes; *NA*, field, cane	UC<CT<BRTP<ISP	a; abc; cd; d
STRAWBERRY *Fragaria x ananassa* 'Redchief'	Leaf spot; *Mycosphaerella fragariae*, field, leaves	UC<CT<BRTP<ISP	a; bc; c; c
	Phomopsis leaf blight; *Phomopsis obscurans*, field, leaves	UC<CT<ISP=BRTP	a; bcd; d; d
	Leaf scorch; *Diplocarpon earlianum*, field, calyx	1st UC<CT<ISP<BRTP	*ns*
		2nd UC<CT<ISP<BRTP	a; bcd; cd; c

Table Appendix B.1 (Cont'd)

Angular leaf spot; *Xanthomonas fragariae*, field, calyx	1st ISP<UC<BRTP<CT	ab; abcd; bcd; d
	2nd UC<ISP=CT<BRTP	a; bc; bc; c
Botrytis gray mold; *Botrytis cinerea*, field, fruit	UC<CT<ISP=BRTP	a; abcd; f; f
Anthracnose fruit rot; *Colletotrichum* spp, field, fruit	UC<CT<ISP=BRTP	ab; bcde; e; e
Tan-brown rot; *Hainesia lythri*, field, fruit	UC<CT<ISP=BRTP	a; abc; d; d
Anthracnose fruit rot; *Colletotrichum* spp., post harvest, fruit	1st UC<CT<BRTP<ISP	abc; cde; de; e
	2nd UC=CT<BRTP<ISP	ab; abcd; cd; d
Botrytis gray mold; *Botrytis cinerea*, post harvest, fruit	1st UC<CT<ISP<BRTP	*ns*
	2nd CT<UC<ISP=BRTP	*ns*
Rhizopus rot; *Rhizopus spp*, post harvest, fruit	1st UC<CT<ISP<BRTP	*ns*
	2nd UC<CT<BRTP<ISP	a; abc; cd; d
Tan-brown rot; *Hainesia lythri*, post harvest, fruit	1st UC=CT<ISP<BRTP	*ns*
	2nd UC<CT<BRTP=ISP	*ns*

Overall Summary for Organic Apple, Cucumber Bioassay, Fruit and Foliar

Based on the results from the CHES organic apple orchard, the particular aerated brewed compost tea and the commercially available product Serenade® did not affect the incidence of apple scab on apple leaves. The bioassay protocol as a test of systemic acquired resistance in cucumber, using 6 different aerated brewed compost tea preparations did not elicit an effect. Results from the experimental field and post harvest spray trials involving a large number of plants and pathogens were sufficiently mixed to warrant contemplation for continued research on the production and use of microbial products based on compost.

A primary outcome was the lack of confidence in the consistency and predictability of the protocol for aerated brewed compost tea. Until positive results can be replicated beyond that of a few controlled studies and antidotal cases, there is a need to expand the range of controllable variables in the production of compost based products which applied suspended in water. Then when positive results are achieved in improvement of plant health, there will be a reproducible process. Proposed recommendations of change are to expand the variables range of temperature, oxygen levels, material composition and maturity of compost, duration, and the media used for cultivation. As previously stated in the literature review all these controllable factors influence microbial development.

The aerated compost tea protocol (Ingham, 2003) states that the water medium temperatures start at 22 °C and remain ambient (not defined) thereafter. We found that due to cooling by the compressed air and ambient night temperatures that fish tank heaters were needed to maintain a consistent temperature. Setting aside the concerns that

the recommendations lack precision and thus the ability to reliably reproduce a replicate product, it is proposed that a system be developed to control media temperature over a wide range so that the selected/favored microbes from the compost and their byproducts can be researched as developed under various temperature settings.

Protocol for aerated compost tea (Ingham, 2003) dictates that the percent of dissolved oxygen in the water based media to be aerobic by remaining above 6 ppm. The three factors that play a significant role in achieving this goal are: 1) the total exchange surface area between bubbles and the media, 2) the amount of available energy to the microbes from the amendment, and 3) the temperature of the media. The pretext for maintaining such oxygen levels is that phytotoxins will not be produced under aerobic conditions. While it may be correct specifically within the water as the media of cultivation, there is an over site in regards to the conditions that are within the device (bag) that contains the compost suspended within the brewer. As the compost is held suspended within the brewer there exists a significant boundary layer affecting the diffusion of oxygen. It has not been determined that compounds produced under such conditions within the compost are not in fact of significant importance. The bioreactor that we developed attempted to address this by maintaining the compost suspended freely in solution. It is proposed that microbial cultivation, using compost as the starting material, over a wide range of oxygen levels be done and researched once this variable can be dictated with confidence.

The protocol calls for that the compost used to meet minimum standards. They are that the duration and range of peak maximum temperature for 3 days to be between 55-68 °C, internal oxygen levels maintained above 12-15% throughout the process, have

the smell of soil, and be slightly immature with an internal temperature 5-10 °C above that of ambient. The parent material used in making the compost is not addressed. It is proposed that reproducible designer compost recipe(s) be developed that are made from materials that are readily accessible and brought together in ratios that ensure a basis for a successful composting process. Once in place the efficacy of various phases of the compost maturity can be tested.

The duration of the brew time, as stated in the protocol, is dependent on a commercial or homemade device. Peak microbial numbers are achieved between 12-72 hours. It is proposed that a system be deployed whereby both the microbial population numbers and identification can be linked with the efficacy of the compost based product.

The last remaining concern is the media used to cultivate the compost based microorganisms. Although the use of water based media has long been favored in various industrial and food productions systems, it may not be optimally suited for the task at hand. Proposed is the use of mature compost as both the inoculant and media in a solid state bioreactor. Using mature compost as the solid state media has distinctive advantages with the broadest and most encompassing attribute being the integrity of the heterogeneous ecological micro sights that remain throughout the process. The microbial population is to be extracted in water at the end of the cultivation period before application to the target sight.

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Appendix C

GC/MS response for tentatively identified compounds taken from an amended mature compost under specific temperature and oxygen settings in a solid phase bioreactor.

Compound A

Treatment (%O_2/ °C)

Hour	3/25	3/40	21/25	21/40
1	0	0	0	0
16.5	0	0	0	259100
24.5	0	5381	0	187160
42.5	0	140540	0	219060
48.5	0	948	0	166330
65.5	0	87269	0	187120
72.5	0	97710	0	33031
89.5	0	54912	-	-
119.5	0	111430	-	-
165	0	296010	-	-
189	ND	245530	-	-

Ion	85
Ion fraction	10.24

Tentative identification

Hexane, 24 - dimethyl-, 3(2H) - Furanone

Table Appendix C.1. The original data for figure 3.6 (Chapter 3). Data are the GC/MS response (total ion count, TIC) for the tentatively identified compound Hexane, 2,4 - dimethyl-, taken from an amended mature compost in a bioreactor with specific temperature and percent oxygen settings. The GC/MS response is calculated from the data for a single unique ion; the fraction that the ion comprises of the total ion count is provided. Each TIC value is the result of dividing the ion count for the unique ion by the ion fraction.