OPTIMIZATION OF OILY SLUDGE COMPOSTING PARAMETERS THROUGH RESPIROMETRY

By

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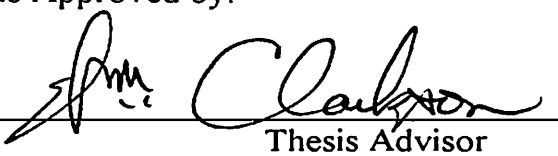

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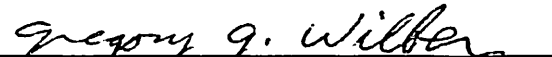

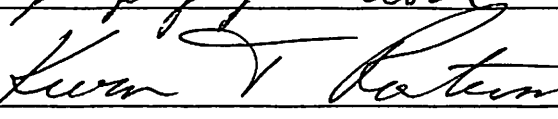

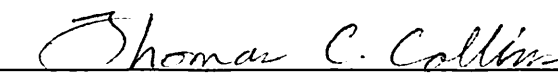

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GLOSSARY

BA	bulking agent
BA/S	bulking agent to sludge ratio (volume/volume unless indicated otherwise)
BOD	biochemical oxygen demand
CC	container capacity (mass water/dry mass material)
CC_{ba}	container capacity of bulking agent (mass water/dry mass material)
CC_c	container capacity of wood chips (mass water/dry mass chips)
CC_S	container capacity of sludge (mass water/dry mass sludge)
DE	diatomaceous earth
DI	de-ionized (water)
dwt	dried weight (mass)
GC	gas chromatography
HC	hydrocarbon
KOH	potassium hydroxide
M	molar concentration (moles/liter)
MC	moisture content (usu. wt%)
O_u	oxygen uptake
psi	pounds per square inch
ppm	parts per million, equivalent to mg/kg or mg/L at low concentrations
S_S	substrate concentration
SR	stoichiometric requirement
VOC	volatile organic carbon
VS	volatile solids

wwt	wet weight
wt%	weight percentage (percentage on a mass basis)
wwt%	wet weight percentage (percentage of total wet mass)
X	cell or biomass concentration
μ	mu, specific growth rate (1/hr.)
µg	microgram = 0.000001 gm

CHAPTER I

INTRODUCTION

The Problem: High Petroleum Content Sludge

For years one of the waste byproducts of the oil industry has been separator and waste pit sludges consisting mainly of crude oils. In the field, these waste oils and blow-down condensates have been conveniently disposed into unlined earthen pits. Over time these waste oils have seeped into the pit bottoms and surrounding soils and achieved high saturations. Other than volatilization of the lighter ends, there often has been little reduction in the mass of hydrocarbons impregnating these soils, even over decades.

Amoco Production Company has over 900 waste oil pits in the area of the San Juan Basin of northwestern New Mexico. Due to the remote location of pits in this region, removal and transportation to reclamation or incineration facilities were considered to be too costly. Amoco recognized that composting of the oily waste pit sludges offered a potentially economical and permanent means of remediation. Other major oil companies had already run pilot composting projects on oily sludges with reported good success (Fyock, *et al.* 1991, McMillen, *et al.* 1992)

Composting as a Bioremedial Solution

Over 200 species of hydrocarbon degrading microorganisms are known to exist. They are found in soils and sediments throughout the world and comprise between one and ten percent of microbial populations in uncontaminated soils. They are capable of using hydrocarbons as their sole source of carbon and energy (Rosenberg and Gutnick,

1981). They are represented in many genera, including *Pseudomonas, Acinetobacter, Flavobacterium, Arthrobacter*, and others. Soil microbes are typically limited in activity by a lack of adequate moisture, nutrients, and source of carbon. When soils become contaminated with oil, the carbon source can be abundant to the point of physically limiting the accessibility of air, moisture, and nutrients, most acutely in soils of low natural permeability. The activity of indigenous oil degrading microorganisms can be greatly enhanced in a composting process by amendment of the limiting factors and physical enhancement of the soil permeability.

Composting is defined as the enhanced natural degradation by microorganisms of an undesirable substance by means of material amendments. Water and nutrients are added to provide an environment for increased microbial growth rates. A bulking material or agent is employed to increase porosity and thus oxygen availability to the microorganisms.

Composting of oily soils and sludges has only recently become documented as a result of feasibility testing in laboratory experiments and pilot projects. Chevron and Exxon, among major oil companies, have conducted research into the composting of oily sludges and have experienced encouraging results. Nordrum, *et al.* (1992) reported a successful project design for a Chevron facility located in Red Wash, Utah. Composting of a sludge with 8% total petroleum hydrocarbons (TPH) resulted in a 97% reduction of hydrocarbons over 41 days. Researchers with Exxon (McMillen, *et al.* 1992) reported a laboratory scale composting experiment that reduced a 10.8% TPH sludge by 92% over four weeks.

Composting offers promise as a viable treatment alternative because it requires relatively low level technology and common materials and is therefore less costly than alternative methods of remediation. Aside from its low cost ($50/yd^3 sludge, according to Nordrum, *et al.* 1992), two other aspects of composting make it a desirable treatment alternative: 1) it is a natural environmental process which is simply enhanced, and 2) it

permanently destroys the offending substrate on site, converting it chiefly to water and carbon dioxide.

A Method for the Application of Composting

The optimum conditions for composting have been difficult to know because of the complex nature of the materials. In some cases composting has failed to degrade the substrate. The heterogeneous makeup of microbial populations and oil contaminants have made for a very complex system of substrate metabolism and growth cycles. Other factors, such as moisture and bulking agent (liquid and solid phase factors) have further complicated the issue. It has not been well understood what relationships exist among various parameters, or even whether the effects among parameters are functionally related or independent of one another. On the other hand, composting *may* be a fairly forgiving process in that optimal conditions may need not be precisely achieved for composting to proceed. In other words, biodegradation may always proceed over fairly broad ranges within some environmental parameters and maybe very narrow ranges in others.

A means of evaluating the varying effects of environmental parameters on the composting process is desirable. Measurement of oxygen uptake, or respirometry, provides a means of quantitatively measuring the microbial activity in compost. Oxygen uptake rate is proportional to microbial activity and population size and therefore to the rate of substrate utilization. Respirometry can provide a measure of the suitability of conditions for microbial degradation of hydrocarbons.

In order to observe the effects of variation in environmental conditions on compost microbial activity, the parameters of hydrocarbon concentration, bulking agent to sludge ratio, moisture content, nutrient concentration, temperature, and compaction were investigated. This was done through a series of tests which were designed to isolate and vary each parameter individually. These tests were conducted on small masses of compost with specific compositions designed to provide data across the practical range of a given

parameter. Samples were tested in pneumatic respirometers where microbial activity was measured and recorded as oxygen uptake data. Tests within a series were compared by two methods: 1) by means of their "steady state" oxygen uptake (respiration) rates and 2) by comparison of growth rates inferred from the early periods of exponential increase in oxygen uptake.

The motive in employing growth and respiration rates to determining the optimal conditions for composting is that microbial rates have singular importance in determining the efficacy of the process. If resulting substrate utilization rates are not sufficient, the duration of time necessary for achieving the desired endpoint may be too great to be practical. (The capability of the composting process to achieve the desired endpoint in terms of final hydrocarbon concentration is an important issue, but not one addressed by this study.) Microbial populations in an environment without limitations in food, nutrient, and other environmental factors will proliferate at exponential rates of growth until something in the environment limits the size of the population. At this point, ideally, the population maintains itself at a steady state and continues to metabolize substrate at some constant rate until the substrate is exhausted (assuming maintenance of other necessary environmental conditions). The level at which the population stabilizes may be dictated by rate limitations in the transfer of oxygen, the availability of moisture, the production of toxic by-products, the exposed surface area of hydrocarbon, etc.

Conceptually, it is desirable to establish *and* maintain as high a population as practical in as short a time as possible. Aside from objective limits, economic and operational constraints may limit the degree to which this can be achieved. For example, it may not be practical to add moisture and nutrients to a static compost pile on a daily basis to maintain a very narrow window of the most optimum conditions.

Knowledge of the conditions most favorable for hydrocarbon degradation in a composting process should ensure a greater degree of success in achieving remediation objectives. This knowlege further can aid in determining maintenance requirements

(moisture and nutrient addition, mixing schedule) and timeliness for completion of the process. In order to gain this knowledge, a methodology for investigation of composting process parameters needs to exist.

Thesis Topic: Suitability of Respirometry for Optimization

The motivation behind this study was to determine whether respirometry is a method suitable for identifying the ranges of environmental parameters which most favor microbial activity in an oily sludge compost. The three main concerns were: 1) whether microbial activity in compost would be sufficient to be measurable as oxygen uptake, 2) whether oxygen uptake would be consistent enough among replicate tests to provide a reasonable level of confidence in their accuracy, and 3) whether oxygen uptake results could be quantified in a way which could provide meaningful comparisons among varying tests. These issues together comprise the topic of this thesis.

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

A literature search was conducted to learn of earlier research into composting and respirometry. The results of those efforts provided background and insight into the composting process and gave indications of accepted values for environmental parameters. A literature review of respirometry provided background on how oxygen uptake data has been interpreted. That knowledge was of critical importance in devising methods for interpretation of the data and for the formulation of new ideas.

Composting

Composting of municipal wastewater treatment plant sludge has been practiced for many years as an alternative to landfarming and landfill disposal. The objectives of sewage sludge composting are: 1) reduction of sludge volume, 2) pathogen eradication, and 3) formation of an odor free, stabilized end product suitable for land application and soil amendment. In 1978 the U.S. E.P.A. developed a forced aeration process employing static compost piles, well known as the "Beltsville" process, which succeeded in achieving the objectives (Nell and Ross, 1987). Since then, the use of various types of composting processes has spread throughout the nation, not only for treatment of sewage sludge, but for other types of municipal refuse and organic industrial wastes.

Application of composting as a means for biologically degrading hydrocarbons has only been documented over the last few years. Taddeo, *et al.* (1989) first demonstrated an in-vessel composting process which degraded 94% of total hydrocarbons present in coal tar, including 84% of priority pollutant polyaromatic hydrocarbons (PAH). Nordrum,

et al. (1992) first documented the field scale composting of a high petroleum content (8%) oil tank bottom sludge, reporting a 97% reduction in 27 major petroleum hydrocarbons (TPH) over 41 days. Kamnikar (1992) reported the successful elimination of low level concentrations (up to 1300 ppm) of gasoline, fuel oil, and diesel from contaminated soils by composting with a mixture of wood chips and manure.

Researchers have investigated some of the individual environmental parameters affecting composting to determine what values or ranges most favor microbial activity. Some of the more commonly investigated parameters have been: 1) bulking agent requirements, both as to type of material and proportion used in relation to soil or sludge, 2) moisture content of compost, 3) temperature, 4) nutrient requirements, particularly as to nitrogen and phosphorus, and 5) porosity and permeability as it is related to type of bulking agent and degree of compaction.

Bulking Agent

Bulking agent to sludge or soil ratio (BA/S) has been perhaps the most commonly considered aspect of compost design. Nell and Ross (1987) reported the use of wood chips in a 2:1 volume ratio to sewage sludge as most effective in a forced ventilation design. Kamnikar (1992) employed a 1:1 mixture of wood chips and manure in a ratio of 1:4 with hydrocarbon contaminated soil in a static, passively ventilated pile. Chevron conducted pilot static pile composting with wood chips at BA/S volume ratios of 4:1, 2.3:1, and 1.5:1 (Fyock, *et al.* 1991). Analytical results were only reported for the 4:1 case which indicated an approximate 90% reduction in TPH. Subsequent field scale up of that operation employed a BA/S ratio of only 1.7:1, perhaps because the sludge was 8% TPH versus 30% in the pilot project (Nordrum, *et al.* 1992 and Martinson, *et al.* 1993). Using bioreactors and respirometers, Stegmann, *et al.* (1991), investigated BA/S ratios ranging from 1:2 to 1:16 (dwt). Aged biowaste compost was used as bulking agent for diesel contaminated soil. They reported the 1:2 ratio as most favorable for microbial

activity and TPH reduction.

Among these and other studies (McMillen, *et al.* 1992, MacGregor, *et al.* 1981), wood chips were the most commonly employed bulking agent. Taddeo, *et al.* (1989), investigated different materials as bulking agents, including wood chips, wood shavings, peat moss, sand, vermiculite, sawdust, and cocoa shells. Biodegradability tests showed that, regardless of material, all tests experienced a 90% reduction of coal tar over an 80 day period. Based upon permeability measurements of compacted compost samples, Taddeo and co-workers selected wood chips as the bulking agent.

Moisture Content

Most studies of the composting process have indicated an optimal value or range of moisture content, as a weight percentage (wt%), considered most favorable for activity. Nell and Ross (1987) suggest a minimum moisture content of 40% for sewage sludge compost and conclude the range of 50% to 60% is optimal. MacGregor, *et al.* (1981) used an initial moisture content of 76% in their sludge composting investigation. Both these research teams indicated that 90% of microbially generated heat is lost through the vaporization of water and suggested the importance of maintaining a water content sufficient to meet that need. Chevron employed a minimum 40% moisture content (MC) in the Red Wash pilot project whereas a minimum of 25% was used in the follow-up field scale operation (Fyock, *et al.* 1991, Nordrum, *et al.* 1992). For an Exxon project, McMillen, *et al.* (1992) used 39% moisture content, described as being 87% of compost "saturation". Stegmann, *et al.* (1992) conducted a series of respirometry tests with diesel contaminated soil compost and identified 60% of "total water capacity" (gm H_2O/gm dry weight compost) as optimal with 50% to 80% as an acceptable range. The notions of "saturation" and "total water capacity" are similar to "container capacity" (CC) as employed later in this thesis. Among these studies, overall moisture contents range from 25 wt% to 76 wt%. Among the researchers, only Stegmann, *et al.* (1992) demonstrated a

method for determining an optimal water content. That work is described later in this chapter within the discussions on respirometry.

Temperature

Temperature is well known to affect microbial activity. Microbial heat output and concurrent air circulation are the primary causes of compost dehydration. None the less, forced air ventilation may be necessary to maintain compost temperatures below a certain incapacitating limit (<60°C) (Hogan, *et al.* (1989). Temperatures in excess of 60°C are necessary for pathogen eradication in sewage sludge compost, but are liable to extinguish hydrocarbon degrading organisms in an oily sludge compost.

Chevron limited temperatures in their pilot scale compost piles by mixing when 135°F (57°C) was reached (Fyock, *et al.* 1991). The pile with a BA/S of 4:1 exceeded 110°F (43°C) whereas the two with lower ratios exceeded 130°F (54°C). TPH reduction was reported for the 4:1 pile at approximately 90%, but was not reported for the other two, leaving open the question of whether the 4:1 pile with the lower temperature threshold achieved the best results. The follow-up field scale compost project also intended to limit temperatures by mixing when they reached 135°F (Nordrum *et al.* 1992). In fact, pile temperature briefly reached 140°F (60°C) and resulted in a 97% reduction in the total of 27 major petroleum constituents.

Taddeo, *et al.* (1989) maintained a temperature of 65 to 85°F (18 to 29°C) within an in-vessel composting system for coal tar. Operating entirely within the mesophilic temperature range, this compost achieved a 94% reduction in TPH over 80 days. Stegmann, *et al.* (1992) conducted respirometry and bioreactor tests on oily compost at temperatures no greater than 30°C and observed significant microbial activity and concurrent reduction in hydrocarbons.

Hogan, *et al.* (1989) conducted bench scale composting experiments at 35°C and 50°C with six specific hydrocarbon compounds, including phenanthrene, fluoranthene, and

pyrene, all amended into a sewage sludge cake and composted for 35 days. Greater hydrocarbon losses were experienced at 35°C than at 50°C, and ranged from 75.1% to 99.7% disappeared. The researchers attributed the result to the greater microbial diversity extant at mesophilic temperatures compared to the thermophilic range.

The three previous studies documented significant hydrocarbon degradation within the mesophilic temperature range of 20°C - 45°C. Two of them reported greater than 90% removal, suggesting that higher temperatures are not required for degradation to occur within a reasonable time frame.

Nutrient Addition

Traditional sewage sludge composting requires the presence of nutrients on the basis of a stoichiometric determination for the conversion of substrate carbon to biomass and CO_2. Nell and Ross, *et al.* (1987) recommend a carbon to nitrogen (C:N) ratio of 30 to 40:1. Haug (1980) favors a C:N ratio of 30:1 or less. In Chevron's pilot scale composting of hydrocarbon contaminated soil an initial nitrogen concentration of 500 ppm was used (0.089 M nitrogen at 40% moisture content). Subsequent additions were made to maintain minimum concentrations of 50 ppm nitrogen and 20 ppm phosphorus (Fyock, *et al.* 1991). The follow up field scale project used an initial dose of 230 ppm nitrogen (0.066 M nitrogen at 25% MC) but also employed manure for 15% of the bulking agent volume. Minimum levels of 50 ppm nitrogen and 20 ppm phosphorus were desired but were difficult to maintain, requiring the addition of up to 230 ppm nitrogen and 160 ppm phosphorus twice weekly. For the composting of a 10.8% TPH sludge, Exxon used an initial nitrogen concentration of 300 ppm urea (0.110 M nitrogen at 39% MC) and 217 ppm phosphate with subsequent maintenance at unspecified levels. Manure was also employed for 14% of the bulking agent volume (McMillen, *et al.* 1992). The manure added to each of the two previously cited examples would have served as an additional source of nitrogen, phosphorus, and other nutrients, as well as serving as an inoculum.

Hydrocarbon Concentration

Sludge and soil hydrocarbon concentrations are factors influencing what BA/S ratio will be employed in a compost. A high TPH sludge has a consistency at room temperature like that of mud or paste and very limited permeability to air. A bulking material or agent is required to effectively increase its air permeability. A lightly contaminated soil has a texture like that of uncontaminated soil and allows some permeability to air. It does not require as much bulking agent to effect an increase in permeability. The quantity of bulking agent employed effectively determines the hydrocarbon concentration in the resulting mixture. Among the all previously cited cases, original soil or sludge TPH concentrations have ranged from a low of around 50 ppm to a high of 300,000 ppm (30%) (Kamnikar, 1992 and Martinson, *et al.* 1993). The 30% TPH sludge employed the highest compost BA/S ratio among the cited studies (4:1). In no study was the hydrocarbon concentration of the resulting compost more than 10% (wt).

Respirometry

Description of Respirometry

Respirometry is the measurement of oxygen consumption by living organisms. In the case of microbial populations, oxygen consumption can be taken as a measure of the population's growth or level of metabolic activity through time and provides an indication of its viability under varying environmental conditions. The most common application of respirometry has been in measuring the five day biochemical oxygen demand (BOD) of wastewaters. The purpose was to provide a measure of the concentration of biodegradable organic material and the microbial culture's ability to consume it. More recently respirometry has been used to measure the biodegradability of specific substances, often to rank them relative to other compounds (Brown, *et al.* 1990; Desai, *et al.* 1990). Respirometry has recently been applied to solid phase materials, such as soils and

composts, to determine conditions which most favorably influence the rate of microbial activity on organic contaminants contained within them (Stegmann, *et al.* 1991).

Theory of Respirometry

An initial premise for use of respirometry as a measure of microbial activity is that a microbial population's size and rate of metabolic activity is proportional to its rate of oxygen consumption. Even when a microbial population is not actively increasing in size it consumes oxygen at some baseline or steady-state level of respiration. A change in the environment, such as in temperature, can act to change that rate of metabolism and thus the rate of oxygen uptake. Oxygen consumption rates also will increase in proportion to the size of a microbial population. A record of oxygen consumption over time can reveal: 1) the rate of initial exponential growth in the microbial population before it becomes growth limited, and 2) the "steady-state" respiration rate which follows a growth limitation and is representative of a population's size and degree of metabolic activity. This "steady-state" rate can be reflective of the severity of an environmental limitation imposed on the microbial community. For example, when all other conditions are constant, a higher "steady-state" oxygen uptake rate observed for one water content compared to another indicates the first water content imposes less restriction on microbial activity.

Exponential Growth Phase. Under conditions of abundant substrate, nutrients, oxygen, and other growth factors, a small starting population of microbes will grow in size at an exponential rate until something in the environment halts its growth. A limitation is not necessarily the depletion of a growth factor, but may be a limitation in the mass transfer rate at which a factor becomes available to the microorganisms. A growth limitation may also be caused by accumulation of toxic byproducts or a lack of available space.

The exponential growth rate of a population is defined by specific growth rate (μ). Specific growth rate is the fractional increase in population or cell concentration (X) per

unit time with respect to its starting size or concentration, expressed in units of t^{-1} as

$$\mu = \frac{dX/dt}{X}. \tag{1}$$

In general, the kinetics governing biodegradation of a non-inhibitory substrate can be characterized by the Monod equation (Metcalf & Eddy, 1979):

$$\mu = \hat{\mu}\frac{S_S}{K_S + S_S}, \tag{2}$$

where $\hat{\mu}$ = maximum specific growth rate ($time^{-1}$),
S_S = substrate concentration (mass/unit volume),
K_S = half saturation coefficient (mass/unit volume).

Grady, *et al.* (1989) presented a methodology for evaluation of the kinetic parameters describing biodegradation in a liquid media by use of respirometry and utilization of oxygen uptake (O_u)as a surrogate measure of microbial growth. He and other researchers recognized oxygen consumption as an energy balance, shown as Equation 3, whereby all the available electrons of a consumed substrate ($S_{so} - S_s$) had to have been transferred to oxygen (O_u) to form carbon dioxide, incorporated into new biomass ($X - X_o$), or ended up in metabolic products ($S_p - S_{po}$).

$$(S_{so} - S_S) = O_u + (X - X_o) + (S_p - S_{po}), \tag{3}$$

where S_s = concentration of soluble substrate (mass/unit volume),
X = biomass concentration (cells/unit volume),
S_p = soluble product concentration (mass/unit volume),
subscript "o" means starting concentration.

Equation 3 can be rearranged to form an expression for oxygen uptake:

$$O_u = (S_{so} - S_S) - (X - X_o) - (S_p - S_{po}). \tag{4}$$

Grady and co-workers recognized that during exponential growth, rates of

substrate removal (dS_s/dt), biomass growth (dX/dt), and metabolic product formation (dS_p/dt) are all proportional to biomass concentration (X), as shown by the following set of differential equations based on the Monod equation (Dang, *et al.* 1989, Grady, *et al.* 1989):

$$\frac{dS_s}{dt} = -\frac{1}{Y}\frac{\hat{\mu}\cdot S_s}{K_s+S_s}X \qquad \text{(rate of substrate removal),} \tag{5}$$

$$\frac{dX}{dt} = \frac{\hat{\mu}\cdot S_s}{K_s+S_s}X - \frac{b\cdot S_s}{K_s+S_s}X \qquad \text{(rate of cell growth),} \tag{6}$$

$$\frac{dS_p}{dt} = Y_p\left(-\frac{dS_s}{dt}\right) = \frac{Y_p}{Y}\frac{\hat{\mu}\cdot S_s}{K_s+S_s}X \qquad \text{(rate of product formation),} \tag{7}$$

where b = biomass decay coefficient (time^{-1}),
Y_p = product yield coefficient (mass products/mass substrate consumed).

Since the three preceding rate expressions are all dependent upon existing biomass concentration, an equation relating those rates would be useful. This is done first by differentiation of Equation 4 with respect to time and results in an expression for rate of oxygen uptake (dO_u/dt),

$$\frac{dO_u}{dt} = \frac{dS_s}{dt} - \frac{dX}{dt} - \frac{dS_p}{dt}. \tag{8}$$

Substitution of Equations 5-7 for the rate terms in the right side of Equation 8 results in the following expression:

$$\frac{dO_u}{dt} = \left(-\frac{1}{Y}\frac{\hat{\mu}\cdot S_s}{K_s+S_s}X\right) - \left(\frac{\hat{\mu}\cdot S_s}{K_s+S_s}X - \frac{b\cdot S_s}{K_s+S_s}X\right) - \left(\frac{Y_p}{Y}\frac{\hat{\mu}\cdot S_s}{K_s+S_s}X\right). \tag{9}$$

Biomass concentration (X) can be factored out to result in

$$\frac{dO_u}{dt} = \left[\left(-\frac{1}{Y}\frac{\hat{\mu}\cdot S_s}{K_s+S_s}\right) - \left(\frac{\hat{\mu}\cdot S_s}{K_s+S_s} - \frac{b\cdot S_s}{K_s+S_s}\right) - \left(\frac{Y_p}{Y}\frac{\hat{\mu}\cdot S_s}{K_s+S_s}\right)\right]X. \tag{10}$$

Thus, it can be concluded that oxygen uptake rate is directly proportional to biomass concentration ($dO_u/dt \propto X$) through the period of exponential growth. Exponential growth occurs only when substrate concentration greatly exceeds biomass concentration ($S_S >> X$) because substrate must be abundantly available to the cells.

Biomass concentration (X) has previously been shown to be proportional to cumulative oxygen consumption (O_u), and likewise dOu/dt proportional to dX/dt (Brown, *et al.* 1990). In fact, during exponential growth, oxygen uptake (O_u), oxygen uptake rate (dO_u/dt), biomass concentration (X) and biomass growth rate (dX/dt) are *all* proportional. This results from the definition of exponential growth, where a change in size is in reference to the starting size ($dX/dt = \mu X$). Thus, when $dX/dt \propto X$, and $dX/dt \propto dO_u/dt$, then $X \propto dO_u/dt$. This reasoning simply verifies that oxygen uptake rate is proportional to the size of a microbial population and can serve as a surrogate for measurement of its growth. It further permits oxygen uptake data to be used for estimation of the kinetic parameters describing a culture's growth.

Precise knowledge of initial substrate (S_{so}) and biomass (X_o) concentrations is required for the kinetic parameters Y and K_s to be estimated. For solid phase mixtures, such as compost, these values are not easily determined. One parameter, however, can be evaluated from oxygen uptake data alone. Aichinger, *et al.* (1992) mentioned a technique for graphically determining a culture's specific growth rate (μ). This was done by plotting its instantaneous oxygen uptake rate against its cumulative uptake, as a continuous function. Instantaneous uptake rate is determined from adjacent cumulative uptake values and is expressed mathematically as:

$$\frac{dO_2}{dt} = \frac{O_{2(t_{n+1})} - O_{2(t_n)}}{t_{n+1} - t_n}. \qquad (11)$$

Aichinger and co-workers used the slope of the initial, linear portion of the uptake rate versus cumulative uptake plot as an approximation of a culture's maximum specific growth rate. In fact, it can be shown that this slope in general is equal to a culture's

specific growth rate. This reasoning can be demonstrated by use of Equation 1 and substitution of $k(dO_u/dt)$ for X (where k is a constant of proportionality):

$$\mu = \frac{dX/dt}{X} = \frac{d(k(dO_u/dt))/dt}{k(dO_u/dt)} = \frac{d(dO_u/dt)}{dO_u}. \quad (12)$$

Equation 12 illustrates two important points: 1) because the proportionality constants cancel out, the resulting oxygen uptake based rate expression is actually equal to a culture's specific growth rate, and 2) a change in oxygen uptake rate with respect to a change in cumulative uptake actually is an expression for the *slope* of a plot of oxygen uptake rate against cumulative uptake. On this type of plot, the exponential growth phase of a culture's life cycle appears linear. The slope of a trend line fitted to that interval is the specific growth rate of the culture (further details are provided in Chapter 3). Recognition of the above two points allows an easy determination of μ for comparisons of culture performance and the biodegradability of various compounds under similar conditions.

Constant Respiration Phase. Aerobic respiration is the oxidation of substrate by living organisms to obtain energy for cell maintenance and growth by use of molecular oxygen as an electron receptor, and which results in the formation of water and carbon dioxide. The period of oxygen uptake following a logarithmic increase to a maximum can be evaluated for its average rate over the interval for which it can be construed to be relatively constant. A constant oxygen uptake rate implies a constant biomass and a constant rate of substrate utilization. Conditions resulting in higher constant respiration (BOD) rates are desirable. Thus, constant BOD rate can be used to compare activities among tests which vary a parameter to determine what values most favor microbial activity. An equation showing the stoichiometry of respiration as utilized by Stegmann, *et al.* (1991) is shown below:

$$C_xH_y + n \cdot O_2 \rightarrow x \cdot CO_2 + y/2 \cdot H_2O + \text{energy}, \quad (13)$$

$$\text{where } n = x + y/4.$$

"Steady-state", constant BOD rate can also be used to determine the rate of substrate utilization and estimate the required time to achieve an endpoint. For this purpose the ratio at which oxygen combines with hydrocarbon is required and may be determined from the above stoichiometry for the oxidation of a selected hydrocarbon (Stegmann, *et al.* 1991).

Cumulative BOD. Five day BOD is too short a time frame to provide meaningful results because compost samples often take up to five days just to initiate measurable exponential growth in a respirometer. Cumulative BOD over the life of a longer term test (approx. 14 days) can provide an indication of the amount of hydrocarbons degraded.

Brief History of Respirometry

The notion of employing oxygen uptake to measure microbial growth is not a new one. In 1890 W. E. Adney reported an effort to measure volumetric changes in the head space over wastewater in a vessel (Jenkins, 1960) . Over the years a series of "manometric" instruments have been devised to measure the decrease in pressure in constant volume reactors. By the mid-1930's two designs, the Warburg and Barcroft respirometers, began to see extensive use for wastewater analysis. Both designs were similar, except that the Barcroft respirometer was a reaction vessel connected via a mercury-filled U-tube manometer to an identical compensation flask containing only an equal volume of water. The intent was to isolate the system from nominal temperature and barometric changes. The Warburg respirometer was not connected to another flask but was open to the atmosphere on the far end of the U-tube and thus was subject to barometric fluctuations. As in present day respirometers, both these designs required the use of a potassium hydroxide (KOH) solution to scavenge produced carbon dioxide. The main limitation of these designs was not the fact that there was no mechanism for providing additional oxygen beyond what existed in the reactor head space. Rather, it was that volumes of sewage tested were limited to a few ml of inoculum in small, 15 ml

reactors because larger amounts would exert a BOD that would exceed the 30 cm range of the manometer (Jenkins, 1964).

By the early 1960's a new type of respirometer had been developed which could deliver oxygen in proportion to its uptake by a culture. The electrolytic respirometer was designed primarily to overcome the limitations of the Warburg and Barcroft types. It was built around a 1 liter reaction vessel connected to a U-tube style chamber of electrolyte. As CO_2 was evolved and absorbed by the KOH in the reactor, it caused the level of electrolyte in its chamber to rise, causing contact between two electrodes, one of which generated oxygen. The oxygen would rise in the head space connected to the reactor and diffuse downwards to the sample fluid. The quantity of oxygen consumed was measured as the time the current was on multiplied by the electrode's known rate of oxygen generation.

The latest development, a computerized pneumatic (or piezometric) respirometer, is significant in its improvements over electrolytic methods. Pressure depletion in the reactors is detected by sensitive computer monitored piezometers connected via tubing to individual air filled reference chambers in a temperature controlled water bath with the reactors. Oxygen delivery is through computer actuated valves and occurs in response to relative pressure depletion in the reactors. Computer recorded uptake data can be gathered at frequent intervals around the clock over extended periods of time. The resulting data are amenable to spreadsheet analysis.

Biodegradability Testing Using Respirometry

A number of researchers have conducted biodegradability studies of individual compounds dissolved in water using respirometry. Respirometry has been used extensively by the U.S. E.P.A. in Cincinnati (Desai, *et al.* 1990) to characterize the biodegradability of compounds in aqueous solutions. This was done by use of estimations of kinetic parameters from oxygen uptake and other data. Dang, *et al.* (1989) estimated

kinetic parameters from oxygen uptake data resulting from the biodegradation of various individual aromatic compounds in solution. Brown, *et al.* (1990) presented kinetic parameter estimates, also derived from oxygen uptake data, for heterogeneous cultures growing on various substituted phenolics. They employed estimates of specific growth rate to rank the compounds in order of their biodegradability. Aichinger, *et al.* (1993) conducted investigations into the biodegradability of low solubility compounds, including four polyaromatic hydrocarbons. They concluded that all kinetic parameters could not be estimated for compounds present above their limit of solubility, with the exception of when the rate of solubilization exceeded the rate of biodegradation. In any case, an estimate of specific growth rate could be made from exponential growth phase oxygen uptake data. This is pertinent because in a wetted, oily compost the solubility and the rate of solubilization of the hydrocarbons into the water phase could place a mass transfer limitation on microbial growth.

The principal efforts of these researchers were directed towards: 1) determining whether their techniques for estimation of kinetic rate constants were valid, and 2) obtaining kinetic rate constant estimates which would allow ranking of the tested compounds in order of their ease of biodegradability. Among the researchers, Dang, *et al.* (1989) alone validated their kinetic parameter estimates by comparison to actual measured rates of substrate removal and biomass growth, demonstrating a favorable comparison with the oxygen surrogate derived kinetic parameter estimates.

Within all studies, specific growth rate (μ) was deemed the easiest kinetic parameter to obtain. This was principally because μ was obtained from early oxygen uptake data, where exponential growth was occurring and no factors were limiting. Specific growth rate was the basis used to rank the compounds in terms of their ease of biodegradability. Cross correlation of the rankings between studies on a relative basis showed a similar, but not identical, ordering. Numerical values for μ were widely dispersed when compared between studies. The discrepancies were attributed to

differences in a microbial community's ability to attack a given substrate (i.e. acclimation). For example, the Brown and Dang groups used acclimated and individually enriched cultures as inoculum, whereas Desai, *et al.* (1990) employed sewage sludge inoculum not acclimated to the presence of the compound of interest. This was reflected in lower overall μ estimates compared to the values determined for the identical compounds analyzed by Brown, *et al.* (1990).

Conditions Necessary for Growth . Based upon these research groups' reports, four requirements emerged as necessary for exponential growth to occur and for the resulting oxygen uptake data to be valid for making kinetic parameter estimations. These requirements are: 1) biomass growth and associated substrate consumption must be the only activities contributing to oxygen uptake, i.e., the biomass must be free of nitrifying bacteria and have a low population of protozoa, 2) all of the biomass must be capable of metabolizing the substrate of interest, thereby eliminating oxygen demand due to endogenous metabolism (Grady, *et al.* 1989), 3) starting microbial populations should be relatively small, thus ensuring that exponential growth can occur, and 4) all factors necessary for growth, such as substrate, nutrients, oxygen, space, etc., must be initially present in abundance. For these points to be realized in some of the forgoing studies, a cultured, acclimated inoculum was used (Brown, *et al.* 1990, Dang, *et al.* 1989). It is believed that microbes indigenous to waste pit sludges meet the first three of the above requirements by virtue of natural selection and acclimatization during their long exposure to sludge, and by their low population counts as measured for this study (100 to 1000 microorganisms per gram).

These studies focused exclusively on the estimation of the kinetic parameters of growth. None of them addressed the possibility of employing an established constant rate of respiration as an evaluatory tool. The nature and design of sample media in continuously stirred batch reactors naturally limits the variety and potential for mass transfer limitations. Only the limited presence of a growth factor in a reactor could result

in an imposed constant respiration rate. Design criteria common to all the previously cited studies required that no single factor be growth limiting. All growth factors were supplied in abundance to ensure maximum exponential rates. In contrast, the abundance of potentially rate limiting factors in compost (water content, surface area, permeability, nutrients, solubility, etc.) provide great opportunity for growth to be restricted and constant rate respiration to result.

Respirometry Testing of Compost. Stegmann, *et al.* (1991) reported on biodegradability testing of diesel-contaminated soil by means of respirometry. Tests were conducted in Sapromat electrolytic respirometers and investigated the parameters of moisture content, bulking agent to soil ratio (BA/S), age of bulking agent and nitrogen addition. The soil was spiked with diesel fuel to 1% by weight. The bulking agent was compost derived from agricultural "biowaste".

Tests varying water content were performed on compost with a BA/S ratio of 1:8, dry weight basis (dwt), for each level of water content. Cumulative oxygen uptake was measured over a four day period and comparisons were based on those values. Maximum uptake occurred at 60% of maximum water capacity. The results suggested to the researchers that 50% to 80% might be an acceptable range.

To evaluate the effect of using biowaste compost of different ages as bulking agent, they were separately mixed with 1 wt% diesel-contaminated soil in a ratio of 1:2 (dwt). The ages of the compost bulking agent were 2, 4, and 6 months. Over a 300 hour period, on a per unit hydrocarbon basis, the six month old compost achieved cumulative oxygen uptake nearly twice that of the four month old compost, which itself was over twice that of the two month old compost. The best result from the oldest compost was attributed to natural selection of microorganisms over time for ability to metabolize humic material, which presumably enables them to better digest hydrocarbons.

In Germany, a commonly used compost (as bulking agent) to soil ratio is 1:9 (dwt). Stegmann and co-workers investigated microbial activity for the ratios 1:16, 1:8,

1:4, and 1:2 (dwt) by respirometry. Soil mass was held constant at 25 g and contained 1% (wt) diesel. Oxygen uptake results showed a clear trend of increasing cumulative uptake with increasing BA/S ratio. The 1:2 ratio incurred the highest cumulative uptake on a per gram hydrocarbon basis.

The above BA/S ratio tests were run in parallel with tests supplemented with 25 mg KNO_3-N. Nitrogen was added on the basis of 1 mg N/gm soil and was also present in the compost used for bulking agent at 0.6 mg N/gm. The tests with added nitrogen exhibited *lower* cumulative uptakes compared to their counterparts without added nitrogen, except for the 0.5:1 BA/S case. Both series exhibited a trend of increasing cumulative uptake with increasing bulking agent proportion, but the rate of increase was greatest in the series with added nutrients.

CHAPTER III

MATERIALS, METHODS, AND EXPERIMENTAL DESIGNS

This chapter presents descriptions of the equipment employed in this research, the methods employed to characterize the experimental materials, those materials' characterizations, and the specific compost designs used to evaluate each of the parameters. The compost design which incorporated the optimal conditions obtained from each of the parameter evaluations is presented and is followed by the procedure used to measure the hydrocarbons remaining in finished tests.

N-Con Respirometers

Principles of Operation

All of the parameter optimization tests were performed in N-Con Systems, Inc. pneumatic respirometers. A respirometer system consists of a computer and a respirometer. The respirometer is an enclosed, temperature controlled water bath containing 12 reactor bays and 12 pressure reference chambers, a pressure differential detection system, and an oxygen delivery system. The computer detects pressure differentials between the reactors and their reference chambers and responds with oxygen deliveries to maintain an equilibrium.

Each reactor has a pressure interface with a reference chamber through external tubing connections and a piezometer. The piezometers detect pressure differentials as little as 0.02" H_2O. When pressure depletion occurs in a reactor relative to its reference chamber, the piezometer signals the computer, which in turn responds with a signal to trigger the reactor's oxygen delivery valve. The delivery valves are solenoid-actuated and

open only momentarily (<1 second). The valves are mounted to a manifold regulated to 10 psi which is connected to a supply of bottled oxygen. Upon respirometer set-up, the delivery valves are individually calibrated to within 1% repeat variability according to a procedure outlined in Appendix A. Valve calibrations overall range from 200 to 250 μg oxygen per delivery.

The reactor vessels are Schott-Duran 500 ml bottles. Each bottle is equipped with an airtight cap, seal and norprene tubing for oxygen delivery. The tubing from each bottle is connected to its respective delivery valve in the manifold. Oxygen delivery results from pressure depletion within a reactor and is caused when carbon dioxide is absorbed by a potassium hydroxide (KOH) solution. The KOH is contained in a small cup suspended in the head space of the reactor. As CO_2 is evolved by microorganisms, it is absorbed by the KOH, causing a pressure decrease in the reactor relative to the reference chamber. The piezometer signals the computer which in turn triggers the solenoid valve, delivering a pulse of oxygen into the supply line of the reactor. This continues as necessary to maintain reactor pressure equivalent to that in the reference.

The computer, operating through N-Con's proprietary software program, Comput-Ox, maintains a simple record of cumulative oxygen consumption. Cumulative uptake throughout this experiment was recorded on an hourly basis. Following the completion of tests, the oxygen uptake records were transferred to another computer for manipulation and analysis.

Special Problems with the Respirometers

A great deal of time was spent troubleshooting and learning about the idiosyncrasies of the respirometers. While most procedures such as calibrations and reactor test preparation were quite straightforward, other operational problems caused much concern and required many hours of attention.

During the course of some early preparatory investigations, it was observed that in

the cumulative oxygen uptake plots there were periodic, simultaneous plateaus occurring within groups of triplicate reactors. Plateaus were also occurring concurrently in other groups of tests within the same respirometer. Each plateau was followed by a brief but greatly accelerated period of oxygen uptake. Even sterile controls were affected, though the degree of the effect was proportional to the activity level of the tests. It became evident that there were leaks in the respirometer system.

In fact, leaks were found to be a significant problem with one respirometer. They were primarily in the reference chamber connections, which were all loose. The effect of reference chambers being in communication with the atmosphere caused the computer to sense a pressure deficit in the reactors whenever barometric pressure increased. The result was a period of accelerated oxygen deliveries as the system responded to achieve equilibrium. This problem was overcome when fittings and connections throughout the respirometer were reset with a thread sealing compound and tested to maintain pressure.

A second significant problem which was never resolved involved the software program designed to run the respirometer tests (Comput-Ox). A frequently recurring memory limitation caused by the program's allocation of internal memory would cause the program to abort and exit to DOS, thereby interrupting all tests taking place in the respirometer. A provision within the program allowed resumption of tests once the program was restarted. An unfortunate consequence of the interruption was a zeroing or reduction of the current cumulative uptake values and the addition of 18 to 22 hours of time to actual elapsed time. Figure 1 shows an example of interrupted data.

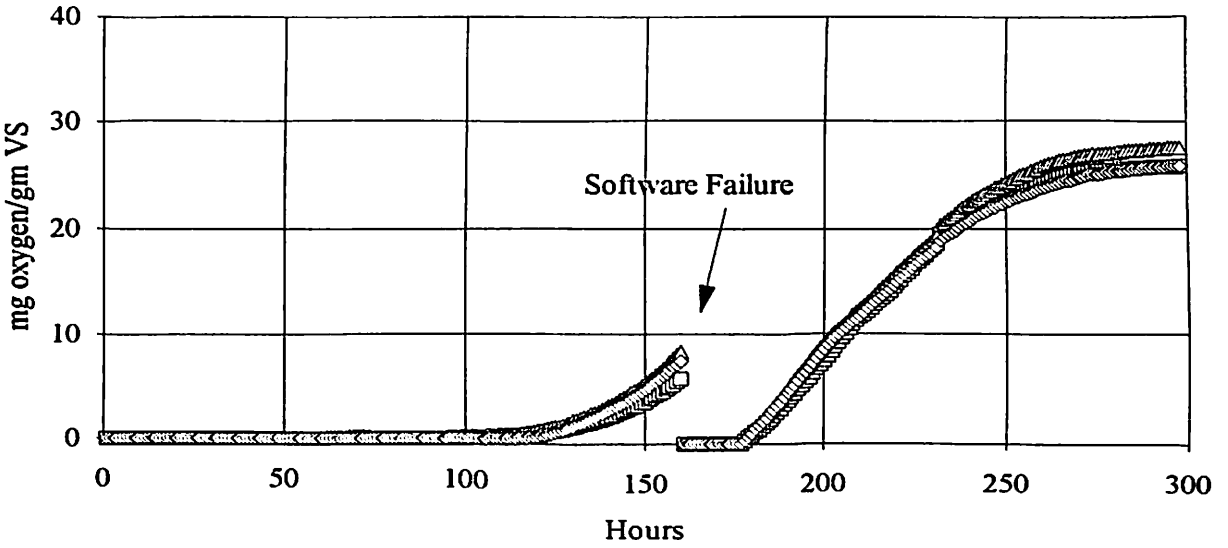

Figure 1. Interrupted Data Set

These events occurred at one time or another in nearly every run, sometimes twice. The memory limitation also caused the program to exit during test start up procedures. This frustrating problem was dealt with using a spreadsheet where data recorded subsequent to a discontinuity could be adjusted to correct values. The Figure 2 shows the same interrupted data set in its restored form. The software failure's impact on the results obtained from restored data was minimal.

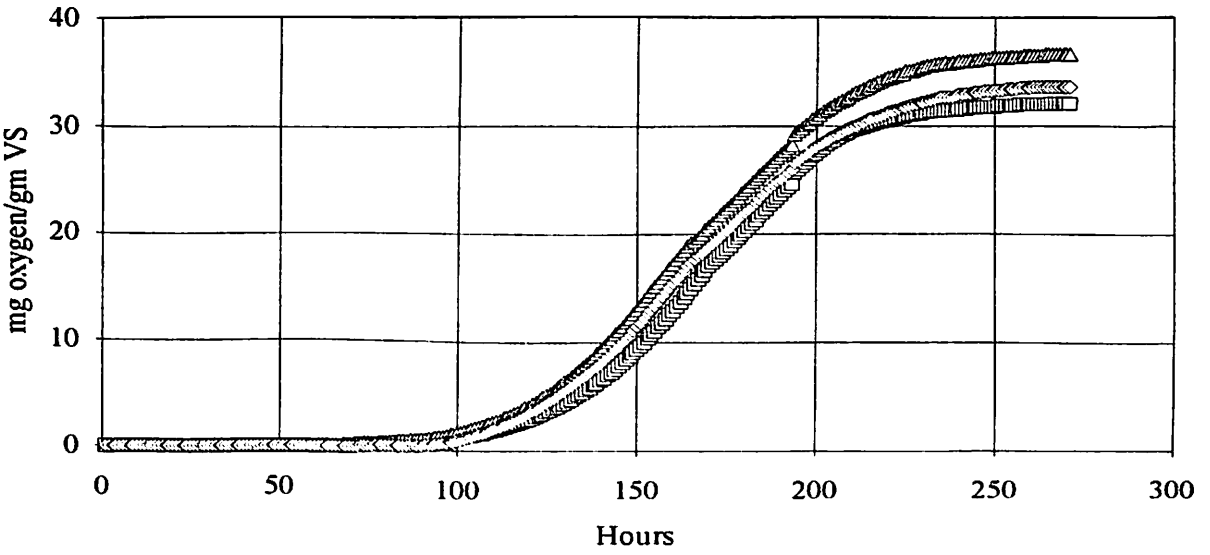

Figure 2. Restored Data Set

Materials Characterizations

Farmington Sludge

The oily sludge obtained for this work was from waste pits located in the area of Farmington, New Mexico. The sludge had originated as a condensate from gas wells which through years of evaporation had lost most of its lighter-end hydrocarbons. Its appearance was like that of a brown paste. The sludge brought to the lab from the field was mixed thoroughly in a tub, divided up into subsamples and sealed. Analysis of sludge content was performed according to the following methods.

Moisture Content. The moisture content of the sludge was measured in a Denver Instruments Company, Inc. moisture balance. The temperature was set to 105°C and the minimum rate of mass loss cut off at 0.05% per minute. Five samples were measured which resulted in a mean moisture mass content of 19.8% ± 0.5%. For the sake of simplicity, the rounded value of 20% was used for design calculations.

Hydrocarbon Content: Total petroleum hydrocarbon (TPH) was determined by two gravimetric methods. In one, the dried sludge remaining from the moisture content analysis was incinerated in ceramic crucibles at 550°C for three hours. The resulting average mass loss of three samples from their *original* wet weight was 24.2% ± 0.5%. The second method was incineration of three fresh sludge samples for total volatiles content (ave = 43.2 ± 1.3%). Average moisture content was subtracted and resulted in an average TPH of 23.6%. The combined average of both methods was 23.9% and was *assumed* to be totally due to hydrocarbons. The rounded value of 24% was used for parameter test design, again for the sake of simplicity.

Hydrocarbons were also extracted from sludge with methylene chloride and analyzed by gas chromatography (GC) for a type analysis. The sludge was initially mixed with diatomaceous earth (DE) at a mass ratio of 4:1 to bind available moisture. The mixture was ground to a very fine texture and extracted with solvent according to a

procedure described at the end of this chapter. Figure 3 depicts the carbon number distribution and mass percentages of the resulting extract as determined by GC. Carbon-23 represents the median hydrocarbon size in terms of carbon number; approximately 47.3% of the molecules had lower carbon numbers, and 47.8% were higher in carbon number. No hydrocarbons less than carbon-9 were detected. More than half (52.4%) of the hydrocarbon molecules present were included in the range of carbon-16 to carbon-25.

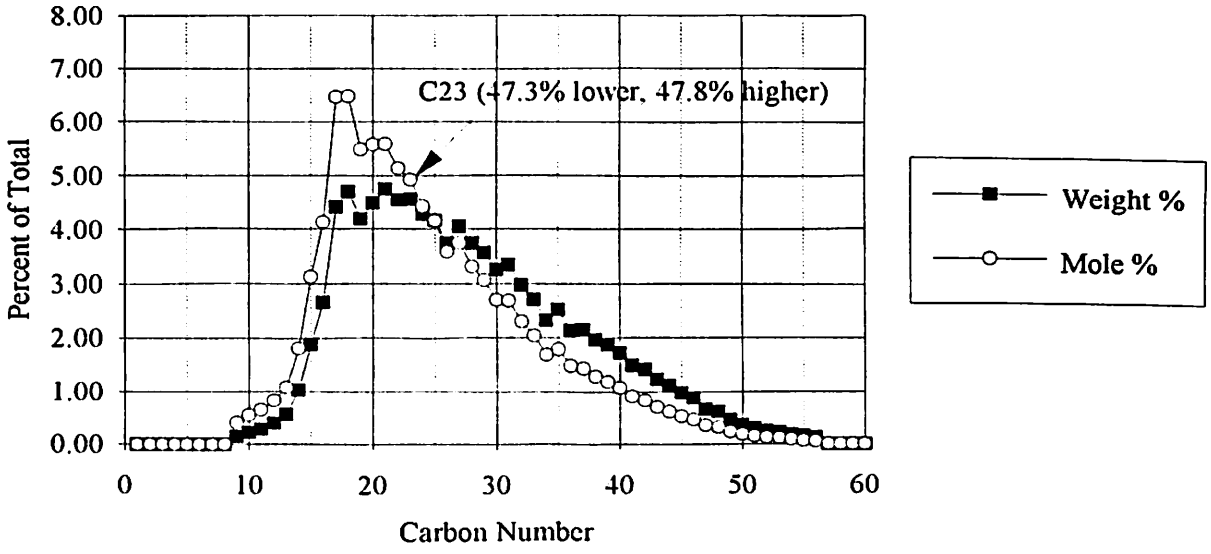

Figure 3. Distribution of Sludge Hydrocarbons by GC

Inert Material. The remaining inert material constituted 56% of the sludge's wet mass. It was comprised of fine to very fine grained quartz sand and clay material.

Microbial Population. Bacterial counts performed by another investigator found bacterial populations in the sludge to be very low, in the range of 100 to 1000 microorganism per gram. However, preliminary tests for sludge activity done in the respirometer were encouraging enough to allow the ensuing experimental work to rely exclusively upon the sludge's indigenous population for hydrocarbon degrading activity. Low starting bacterial populations almost ensure an exponential growth period.

Bulking Agent

The selection of a bulking agent material was a matter of some concern. It was reasoned that it should have all the physical characteristics of a natural material as would be employed in the field (texture, compressibility, size distribution). Chips made from 1" x 2" x 8' long white pine wood strips processed through a chipper were selected for the reason of maintaining consistency in the material.

Wood chips which had been subjected to solvent extraction (described below) contained 0.33% inert material and thus were 99.7% volatile. This was determined by incineration of samples in a furnace at 600°C for three hours. The density of the raw wood chips was determined to be 0.20 gm/cm^3 by measurement of the mass of one liter of loosely compacted chips. Solvent-extracted chips were measured in a likewise fashion with a resulting density of 0.17 gm/cm^3. This value included an average background moisture content of 12%. Moisture contents of the extracted chips were measured before use in a Denver Instruments, Inc. moisture balance and varied from 6 to 10%. The size distribution of the wood chips is depicted in Figure 4 and was determined from the sorting of 300 grams of chips for 20 minutes in a Ro-Tap sorter. The container capacity of the solvent extracted chips was 2.96 gm H_2O/gm dwt. (see Appendix A for method).

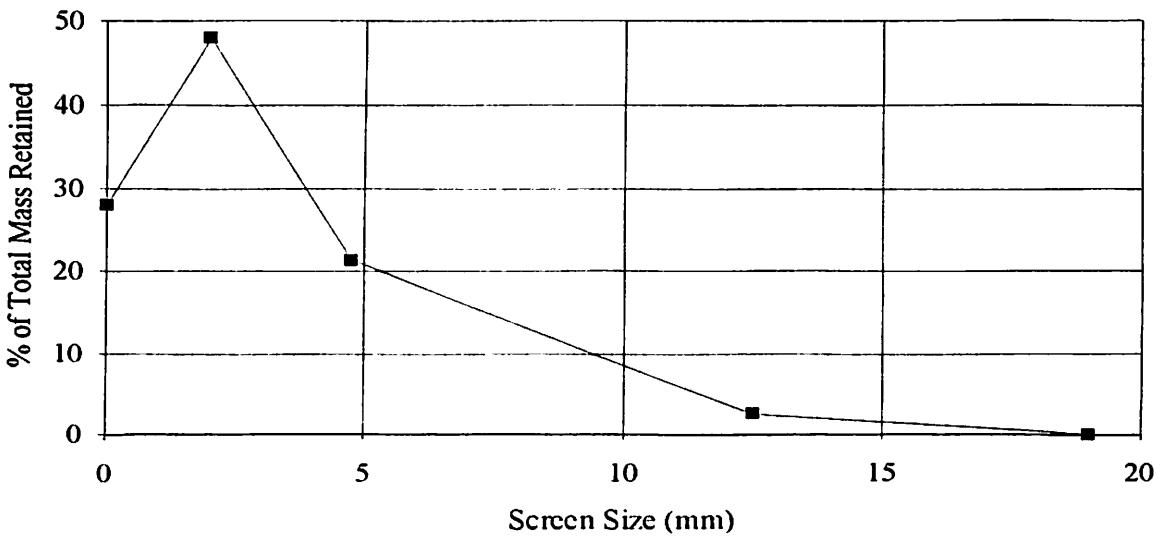

Figure 4. Pine Wood Chip Size Distribution

Preliminary tests in the respirometer with an inoculum derived from the sludge showed that a great deal of microbial activity occurred on raw wood chips. As a consequence, chips were subjected to solvent extraction in 1.75 liter capacity Soxlhet vessels to remove oils which could serve as substrate for microorganisms. Two solvents were used in sequence for 24 hours each, first methanol (polar) and secondly chloroform (non-polar). The resulting chips were termed "double extracted". After extraction the chips were spread and air dried beneath a lab hood for 24 hours and next packed into 250 ml containers and sealed with foil (see Appendix A for details).

Twenty-four hours prior to respirometry testing the chips were sterilized in a Harvard/LTE Benchtop 90 autoclave with a sterilization cycle of 30 minutes at a minimum of 120°C. Fast cooling in the autoclave was desirable to achieve a consistent moisture content in the range of 6% to 10%. On one occasion, inadvertent use of slow cycle cooling resulted in a moisture content of 18%. Figure 5 shows the activity of a sludge derived inoculum on the double extracted chips versus that on the non-extracted chips. Extraction delayed the initiation of significant activity from 25 hours to 70 hours and sharply reduced the initial rates of oxygen uptake. Additional discussion of microbial activity on wood chips compared to sludge hydrocarbons can be found in Appendix A.

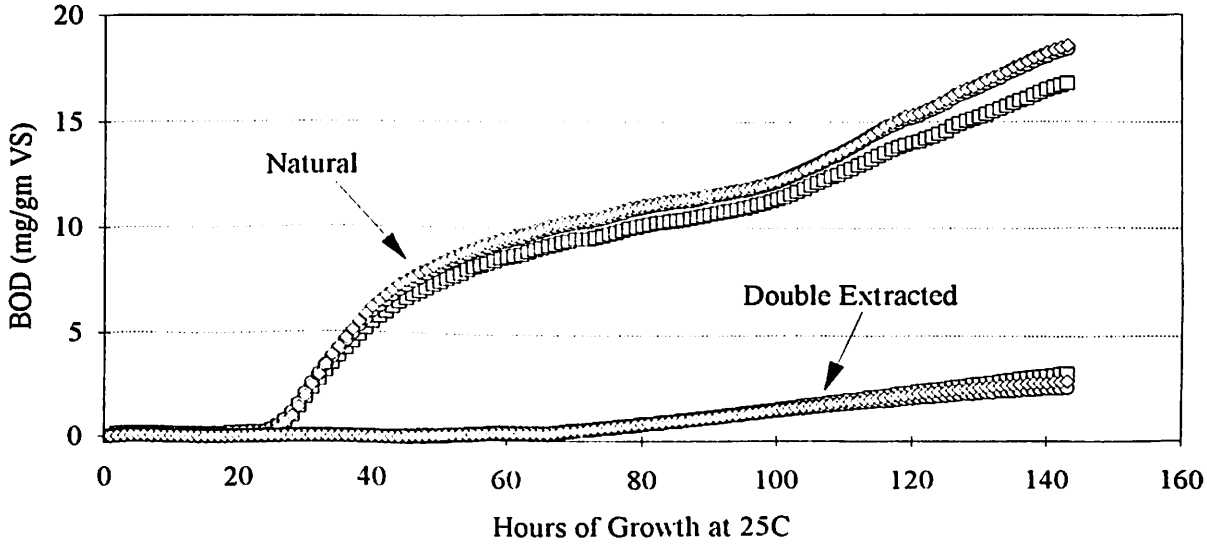

Figure 5. Microbial Activity on Wood Chips

Nutrient Salts Solution

Mineral nutrient supplements are commonly employed to instigate higher rates of bacterial growth than might otherwise be realized in their absence. To ensure that tests were not totally devoid of nutrients a minimal salts medium in the form of a liquid supplement was added to all series of tests.

The nutrient supplement was a combination of a mineral salts media (Evans, 1965) and a trace element solution (Drews, 1974). Evans' mineral salts media had originally been concocted for culturing *Pseudomonas* strains of hydrocarbon-degrading soil bacteria on anthracene and phenanthrene. It consisted of salts containing the most important growth requirements: nitrogen, phosphorus, sulfur, magnesium, and iron. Drews' trace element solution contained a host of trace metals and nutrients necessary for growth. Both Evans' and Drews' formulations are shown in Appendix A.

A standard solution of Evans" media contains nitrogen as ammonium in a concentration of 0.01515 moles/liter. Since nitrogen is the element among nutrients which is required in greatest amounts, its concentration in the form of ammonium was used as a "yardstick" measure for the entire nutrient solution. All stoichiometric requirements were calculated solely on the basis of the nitrogen requirement. Phosphorus is commonly acknowledged to be required at about 20% of the mass of nitrogen (Metcalf and Eddy, 1979, Grady and Lim, 1980). In Evan's solution, phosphorus is present at 84% of the mass of nitrogen. All other elements remained in constant proportion to the amount of ammonium added to a test.

In some series of tests, such as the one investigating the effect of increased nutrient concentration on growth rates, it was necessary to add more standard nutrient solution than could be added as liquid due to the constraint of a limiting water content. Two concentrated solutions of the nutrient media were prepared to circumvent that problem, one at 0.758 molar ammonium (50X standard) and the other at 2.41 molar (159X standard). All other constituents were proportionally present as in the standard solution.

These two solutions were supersaturated and had to be stirred to suspend the precipitate. In most cases, precipitate dissolved when added to whatever makeup water was being used for a batch preparation.

Oxygen Supply

Commercial available bottled oxygen was used for the respirometer supply. It was high purity (HP) grade, being 99.6% pure. Industrial grade oxygen was not suitable for this research.

Test Preparation

Sterilization measures were taken to ensure the only microbes introduced into the reactor bottles were the ones indigenous to the sludge. All cleaned reactor bottles were sterilized in an Harvard/LTE Benchtop 90 autoclave at 120°C for 15 minutes and were stored with regular bottle caps on. Other mix containers and implements were covered or wrapped in foil and sterilized in like fashion. Wood chips were sterilized at 120°C for 30 minutes to assure heat penetration to the core of the foil covered 250 ml containers. The one liter nutrient solution and de-ionized make up water bottles were also sterilized in the same way. Both these liquids were normally issued from repipet dispensers which had been first flushed and purged with sterile solution. All implements used to load sludge and mix compost were foil wrapped and sterilized in preparation for use.

A particular order was followed in the make up and mixing of compost batches for respirometer tests. A typical compost batch consisted of sufficient material for three replicate reactors and was prepared in an 800 ml disposable plastic container. First the nutrient media and supplemental water were added and mixed to ensure dissolution of any undissolved solids in the nutrient. This was followed secondly by the addition and mixing of the chips to ensure they would absorb the available water (sludge coated chips have difficulty absorbing high amounts of water). Sludge was added last of all to the mixture.

A complete and thorough mixing was considered very important and took three to four minutes to achieve. Once well mixed, the compost was loaded into reactor bottles one at a time and the resulting net mass in each recorded. Masses were usually within a few grams (±5%) of being equal.

Potassium hydroxide (KOH) pellets placed in small cups suspended in the head space of the reactors were used as the CO_2 scavenger. Because they were strongly hygroscopic, the pellets were wetted with 2 ml of de-ionized water to eliminate drying of the samples. Some preliminary respirometry tests done with dry KOH dried 10.0 gram samples of flattened sludge to a crust after a week's time. Subsequent tests with 10.0 grams water in each of twelve reactors, half with dry KOH and half wetted with 2 ml water, proved that dry KOH absorbed twice as much water as the wetted over a 44 hour period (1.3 vs. 0.65 gm). As a result of this, all further tests employed KOH wetted with 2 ml of de-ionized (DI) water. In addition, another KOH cup containing only DI water was suspended beneath the first to serve as a supplemental source of head space moisture. In later tests, the second cup was also dedicated to KOH to allow longer run times Generally, on the basis of a one-for-one evolution of CO_2 from biologically consumed O_2, 4.0 grams of 88% purity KOH are required for each gram of total oxygen consumption (see Appendix A for details on the stoichiometry of the KOH reaction with CO_2).

Tests run at elevated temperatures were found to also affect the amount of moisture absorbed by the wetted KOH. Among identical tests, those run at 40°C had their KOH cups filled to capacity after two weeks run time compared to half full cups for those run at 25°C. Both groups of tests had consumed nearly identical amounts of oxygen and therefore evolved equivalent amounts of CO_2.

Run times for tests initially were on the order of 180 hours. Later it became apparent, especially for tests run with elevated concentrations of nutrient, that longer periods of time were required to observe peaking of oxygen uptake rates and the following periods of constant rate oxygen uptake. Most later tests were run for two

weeks (336 hours). This seemed to coincide also with the cup capacity limit for water and CO_2 absorption by KOH.

Hydrocarbon Concentration Tests

The first matter for investigation was that of how overall hydrocarbon (HC) concentration in compost material affects microbial rates. For hydrocarbon concentration to be variable through a series of tests, its mass in the compost mixture samples had to change from test to test while all other constituents remained constant. A base condition was established at a bulking agent to sludge ratio of 2.86 to 1 on the basis of dried weight of wood chips to mass of sludge inert solids. Sludge inert solids were used as the denominator since hydrocarbon mass had to vary throughout the tests. The 2.86/1 ratio was equivalent to a 2/1 mass ratio when hydrocarbons were included. Since the concentration of hydrocarbons in the sludge was 24%, the resulting concentration in the base condition compost mixture was 10% on a dry weight basis (dwt). Higher concentrations were achieved by amendment with hydrocarbons obtained by Soxhlet extraction of sludge (described at end of chapter). Lower concentrations were achieved by amendment with inert material derived from incineration of sludge at 600°C.

In addition to maintaining the masses of inert sludge material (5.60 gm) and bulking agent (16.00 gm dwt) constant, the nutrient and moisture contents were also held constant throughout the tests. Moisture content was arbitrarily chosen to be 62% with respect to the mass of inert and chip material. These were used since they were constant masses, whereas the mass of hydrocarbons varied from test to test. The partial composition of individual tests is shown in Table 1. A complete test make-up is shown in Appendix B.

TABLE 1

PARTIAL MAKE-UP OF HC CONCENTRATION TESTS
(in grams)

	TEST #	Sludge	Added Inerts	Added HC	Total HC	% HC (dwt)[a]
Series 1	337p72	0.00	5.60	0.00	0.00	0.0%
	337p82	2.31	4.30	0.00	0.55	2.5%
	337p73	4.74	2.95	0.00	1.14	5.0%
	337p83	7.30	1.51	0.00	1.75	7.5%
	337p70[c]	10.00	0.00	0.00	2.40	**10.0%**
	337p74	10.00	0.00	1.41	3.81	15.0%
	337p71	10.00	0.00	3.00	5.40	20.0%
Series 2	337p104	0.00	5.63	0.00	0.00	0.0%
	337p94	0.45	5.34	0.00	0.11	0.5%
	337p93	0.91	5.08	0.00	0.22	1.0%
	337p92	2.31	4.30	0.00	0.55	2.5%
	337p91	4.74	2.94	0.00	1.14	5.0%

[a] % HC dwt is with respect to HC, inert solids and chips (dwt).
[b] % HC in Inerts is w.r.t. HC and inert solids only.
[c] Base condition

The term "volatile solids" (VS) includes the mass of chips (dwt) and the mass of hydrocarbons in the sludge; essentially the total mass of potentially biodegradable material. Volatile solids was defined and used in this way because the actual mass of HC in this series is very small at low concentrations. Considering the backgound level of activity on wood chips and its large presence compared to hydrocarbons, oxygen uptake would appear artificially high if measured on a per unit mass HC basis alone. Oxygen uptake throughout the parameter tests was measured on the basis of uptake per unit mass VS.

Bulking Agent to Sludge Ratio Tests

The intent of this series of tests initially was to investigate the effect of varying the bulking agent to sludge (BA/S) ratio on rates of microbial growth and respiration. Knowing that a certain level of activity would occur on whatever mass of wood chips was

present, the design was based on the employment of a constant mass of chips through the series. Thus, only the amount of sludge was varied to achieve different BA/S ratios. Since oxygen consumption in the respirometers was expressed on the basis of concentration (mg O_2/gm VS) rather than simply as total oxygen consumed, differences between the rates could be attributed to changes in the amount of sludge relative to chips.

The range of the first series of tests bracketed the base condition BA/S ratio used in the previous HC concentration tests, that is, 2.86 to 1 on the basis of dry weight to inert solids. This basis was used to be consistent with the previous series. The dry mass of wood chips used was 16.00 grams, except for the last two tests of the third series where the compost volume became a limiting factor. In those tests, the mass of chips was reduced relative to the mass of sludge.

In the previous concentration series, moisture content was related to sludge inert solids and chip mass, both of which were held constant. In this series, sludge inert solids varied with sludge mass so moisture content was tied to the mass of chips, the only solid held constant through the series. Thus, added moisture was chosen near the midrange (50%) of wood chip container capacity (CC_c). Container capacity is defined as the ratio of the mass of water retained by a material against gravity drainage to the dried mass of the material present (Cassel & Nielsen, 1986). See Appendix A for details on its determination.

The only added liquid was Evans' media which was constant at 23.67 gm of the standard solution (0.0152 M ammonia). Since the "sludge only" case had no chips, 0.95 grams of 0.379 M (25X) Evans' concentrate was used to achieve the same stoichiometric amount of nutrient addition. All tests were run in triplicate. Table 2 shows the make up of the variable portion of the BA/S ratio tests. Following the first series of tests, two additional series extended the range of the tests both above and below the original range. More detailed makeup data can be found in Appendix B.

The second series was designed specifically to investigate very low hydrocarbon

concentrations. It overlapped into the range of the first series and repeated the 5% HC concentration test. The third series investigated very low bulking agent to sludge ratios and repeated the 20% HC test.

TABLE 2

MAKE-UP OF BULKING AGENT TO SLUDGE RATIO TESTS
(in grams)

	Test #	Mass Chips (dwt)	Sludge (wwt)	Mass HC	% HC (dwt basis)	Total Moisture	Moisture Content (%)	BA/S Ratio (dwt/ inert basis)	BA/S Ratio (vol/vol)
Series 1	337p97	16.00	4.00	0.96	5.0	25.67	57%	7.14	32.9
	337p98	16.00	10.00	2.40	10.0	26.87	53%	2.86	13.2
	337p99	16.00	20.00	4.80	15.0	28.87	47%	1.43	6.6
	337p100	16.00	40.00	9.60	20.0	32.87	41%	0.71	3.3
	337p102	0	176.23[b]	42.30	30.0	36.20	20%	0/1	0/1
Series 2	361p22	16.00	0.69	0.17	1.0	25.01	60%	41.0	191
	361p21	16.00	1.82	0.44	2.5	25.23	59%	15.7	72.4
	361p20	16.00	4.00	0.96	5.0	25.67	57%	7.14	32.9
	361p19	16.00	6.67	1.60	7.5	26.20	55%	4.28	19.8
Series 3	361p40	16.00	40.00	9.60	20.0	32.87	41%	0.71	3.3
	361p41	16.00	60.79	14.59	22.6	37.03	36%	0.47	2.2
	361p42	16.00	119.04	28.57	25.7	48.68	30%	0.24	1.1
	361p44	12.00[a]	119.04	28.57	26.6	48.38	31%	0.18	0.83
	361p45	8.00[a]	119.04	28.57	27.7	48.08	32%	0.12	0.55

[a] had decreased mass of chips rather than increased mass of sludge due to volume constraints.
[b] to approximate the volume of other tests in the series.

Water Content Tests

To evaluate the effect of moisture content on the microbial activity of a compost mixture, a practical range of moisture contents had to be tested. In the absence of any added water, compost is a mixture of just two materials, the bulking agent and the sludge. The natural moisture content of each could provide a convenient starting point for moisture content investigations.

To determine the practical high end limit for moisture content, the concept of container capacity (CC) was employed as outlined in *Methods of Soil Analysis* (Klute, 1986). Briefly, container capacity of a material is determined by placing it into a container with a perforated bottom and known mass. The container is set in a shallow pan of water and allowed to wet by capillary rise for 12 hours, during which time it is gradually immersed to its top to saturation. After immersion the container is covered and allowed to drain by gravity for six hours. The resulting mass of the material and held water is determined. Next, the container is oven dried for 24 hours at 105°C, after which the dried mass of the material is determined. The net loss in weight represents the previously held water. This water mass divided by the mass of the dried material is its container capacity (gm H_2O/gm dwt material). Thus, container capacity represents the amount of water that a material can retain after gravity drainage for six hours. Appendix A contains a more detailed description of the procedure.

Initially, container capacity was measured for both the sludge and the wood chips individually. The sludge exhibited negligible drainage with a $CC_S = 0.22$ (nearly its moisture content, 20%), whereas the double extracted pine wood chips retained a high mass of water with $CC_C = 2.96$. In comparison, raw, non-extracted wood chips had a container capacity of 0.69.

The BA/S ratio employed for the moisture content series of tests was 2.86/1 on a dry weight to inert solids basis (as employed in the HC concentration series). The container capacity for this compost mixture was measured at 1.58, significantly less than the chips by themselves. The base condition of the mixture then became the natural moisture contents of the sludge and chips, equated to percentage of compost container capacity. The range of moisture levels up to 100% of chip container capacity was tested. This resulted in some tests actually exceeding compost container capacity. In these tests, water present in excess of compost container capacity was present as free water at the bottom of the container and represents that water which would drain away from an overly

wetted compost pile. Moisture test make up is shown in Table 3.

TABLE 3

MAKE-UP OF MOISTURE TESTS

(in grams)

TEST #	% CC Chips	% CC Compost	% Moisture Content	Sludge	% HC (dwt)	Chips (wwt)[a]	Evans' Conc.[b]	Add'l Water
361p2	5.3	12	16	10.00	10.0	17.52	1.00	0.00
361p3	33	46	43	10.00	10.0	17.52	1.00	13.28
361p4	50	67	52	10.00	10.0	17.52	1.00	21.20
361p5	67	88	58	10.00	10.0	17.52	1.00	29.12
361p6	83	108	63	10.00	10.0	17.52	1.00	36.99
361p7	100	129	67	10.00	10.0	17.52	1.00	44.91

[a] Chip dry weight equals 16.00 gm
[b] Evans' concentrate is 0.758 molar (50X standard)

As can be seen from the preceding table, amounts of sludge, chips and nutrient all remain constant. Only the water content varies. The mass of de-ionized water (X_w) to add to achieve a certain moisture content was determined by use of the equation:

$$X_w = \frac{m_s(MC - MC_s) + m_c(MC - MC_c)}{1 - MC} \tag{14}$$

where: m_s = mass sludge
m_c = wet mass chips
MC_s = moisture content sludge
MC_c = moisture content chips
MC = desired overall moisture content (all MC's in decimal form)

Nutrient Addition Tests

The amount of nutrient elements available to a microbial community certainly affects its rate of growth. This series of tests was designed to observe the degree to which growth rates could be influenced by different levels of nutrient availability. Since microbes

live in the aqueous phase, nutrient availability was defined in terms of its concentration in the total available water.

The principal nutrient necessary for bacterial synthesis in terms of its relatively high stoichiometric requirement is nitrogen, principally in the form of ammonia. Nitrogen's importance is obvious by its presence in the empirical formula for bacteria, $C_5H_7O_2N$. Next in importance is phosphorus, which is commonly present in a ratio of one phosphorus to twelve nitrogen on a stoichiometric basis. A host of other minerals and trace elements are necessary for optimal growth. A combined solution of Evans' mineral salts media and Drews' trace element solution was employed for this series of tests (see Appendix A for the formulations of Evans' and Drews' solutions).

The basis for determining the quantity of nutrient addition was the stoichiometric requirement for nitrogen necessary to completely convert the available hydrocarbon carbon to biomass. This calculation was dependent first upon an assumed 85% carbon mass content for the hydrocarbons in the sludge. Complete conversion of the carbon to biomass was of course a gross assumption representing the absolute upper limit. Stegmann, *et al.* (1991) employed a C:N:P ratio of 73.2:9.2:1 for their experiments with composting of oil-contaminated soil in bioreactors. This ratio was employed here in initially determining what the stoichiometric requirement for nitrogen and phosphorus would be for the 2.40 grams of hydrocarbon contained in ten grams of sludge. The quantity of standard Evans' media necessary to provide the requirement was 1.410 liters for nitrogen versus 0.405 liters for phosphorus. For this reason a 50X concentrate was prepared. Nitrogen in the form of ammonium was used as the "yardstick" for determining levels of nutrient addition. Addition was scaled over the range of none to full addition of the stoichiometric requirement for nitrogen based on Stegmann's C:N ratio (~8:1) for conversion to biomass.

The design of these tests employed constant amounts of sludge and wood chips at the usual BA/S ratio of 2.86/1 (dwt to inert). The requisite amount of nutrient

concentrate was supplemented by sufficient sterile, de-ionized water to bring the overall moisture content to 58% by weight. Initially, four tests equally divided the full range of nutrient investigation. Following results of that group, four additional tests were run bracketing the lower one third of the range. Triplicates for all tests were assembled as shown in Table 4.

TABLE 4

MAKE-UP OF NUTRIENT ADDITION TESTS
(in grams)

	Test #	Added NH_4^+ (% Stoich. Req.)	Sludge Mass	Chips (wwt)[a]	Added Nutrient[b]	Added Water	Total Water[c]	Resulting NH_4^+ Conc. (M)
Series 1:	337p87	0.00	10.00	17.35	0.00	30.30	33.65	0.000
	337p88	33.30	10.00	17.35	10.03	20.90	33.71	0.225
	337p89	66.60	10.00	17.35	20.06	11.50	33.77	0.450
	337p90	100.00	10.00	17.35	30.09	2.10	33.84	0.674
Series 2:	337p105	8.30	10.00	17.69	2.51	27.61	33.67	0.056
	337p106	16.60	10.00	17.69	5.02	25.26	33.69	0.113
	337p107	25.00	10.00	17.69	7.52	22.91	33.69	0.169
	337p108	33.30	10.00	17.69	10.03	20.56	33.71	0.225
Series 3:	361p08	0.00	10.00	17.40	0.00	30.24	33.64	0.000
(repeat	361p09	33.30	10.00	17.40	10.03	20.84	33.70	0.225
of 1)	361p10	66.70	10.00	17.40	20.06	11.44	33.76	0.450
	361p11	100.00	10.00	17.40	30.09	2.04	33.83	0.674

[a] Dried weight wood chips is 16.00 gm
[b] Evans' concentrate density is 1.06 gm/ml
[c] Total moisture = sludge water (2.00 gm), chip water (1.40 to 1.69 gm), nutrient concentrate/1.06, and added water.

Temperature Tests

Temperature is well known to effect rates of microbial growth. In general, two temperature intervals, the mesophilic and the thermophilic ranges, foster the highest rates of bacterial growth. Certain bacteria are viable only in one particular temperature range or the other. The mesophilic range extends broadly from 10° to 45°C. The optimal segment

of that range is between 25° and 40°C. The thermophilic range of elevated growth rates exists broadly from 45° to 75°C. The optimal interval is from 55° to 65°C (Metcalf and Eddy, 1979). All previous series of tests for this project were run at 25°C. Since composting activity generally generates an excess of heat and increased microbial rates could be expected to occur at elevated temperatures, a series of tests was designed to measure activities at temperatures above 25°C.

A practical limit was placed on the high end of the range of investigation by a design flaw in the water bath hood of the respirometer itself. At 45° and 50°C temperatures large volumes of condensation on the interior of the hood became a concern. In the absence of guttering to direct condensation back into the bath, water trickled over the side, into the control panel and onto the floor.

Another problem which became common at the higher operating temperatures was caused anytime the hoods were raised, even for as little as 10 seconds. The cooling effected by the 20°C ambient room air set off a flurry of oxygen deliveries as the gas in the reactor hoses contracted.

For each temperature, two tests were run, each at a different level of nutrient addition. One was with standard Evans' media supplied as the sole moisture supplement to the compost mixture. This resulted in an overall 0.014 molar ammonia concentration for the batch. A higher level of ammonia concentration was chosen in the optimum specific growth rate range observed in the nutrient addition series of tests. That amount (17% of the stoichiometric requirement for nitrogen) resulted in a molar ammonia concentration of 0.112 for the batch. The intent of the design was to maintain moisture content at 58% by weight (67% of CC_C) as in the nutrient series. However, a mistake was made and moisture content was set to *67%*. Tests for each temperature were set up as shown below in Table 5. Sets of sterile controls were run to ensure that chemical oxidation was not responsible for oxygen demand.

TABLE 5

MAKE-UP OF TEMPERATURE TESTS
(in grams)

% Stoich. Req. Nitrogen	Sludge[a]	Chips (wwt)	Standard Evans'[b]	Evans' Conc.[c]	Added Water	Resulting NH_4^+ Molar Conc.
3.2%	10.00	17.58	44.49	0	0	0.014
25%	10.00	17.58	0	7.52	37.40	0.112
Sterile Control	10.00	17.58	0	7.52	37.40	0.112

[a] Sludge contains 20% water.
[b] Standard Evans' media is 0.0152 M ammonium.
[c] Evans' Concentrate is 0.758 M ammonium and has a density of 1.06 gm/ml

Temperature tests were run at 35, 40, 45 and 50 degrees Centigrade. Results from tests run in other series at 25° C were used for comparison. The high nutrient test from the nutrient investigation series (337p106) could not be employed for comparison because of its different moisture content of 58% . One of the low nutrient tests was from the moisture content series (361p7). It used 1.00 ml of a concentrate for nutrient amendment rather than a dilute form which resulted in ammonium being at 3.55% rather than at 3.2% of the stoichiometric requirement. 361p7 was later repeated as 361p53 and employed the dilute nutrient media instead.

Compaction Tests

Compaction was investigated for its impact on microbial rates. It was anticipated that the principle result of compaction would be a lessening of the porosity and permeability of the compost matrix and a reduction in the availability of oxygen to the microbial community. Compaction is a real consideration in the design of compost piles and places limitations on the height to which they can be built.

In order to conduct these tests, a different style of respirometer reactor vessel was required. One liter, straight sided, wide mouthed bottles were used. Compaction was

achieved by compressing different measured masses of compost to one particular volume. In order to compress the compost, rigid stainless steel screens were set on top of the compost, then 5" spacers cut from 2 1/2" diameter PVC pipe were placed on top of the screens and pressed down by the caps as they were screwed into place.

Make up of the tests is shown in Table 6. The constant volume achieved through compaction was 250 ml. This volume was selected on the basis of maintaining the average height of compost material observed in the bulking agent to sludge ratio tests. (Those tests were used for 0% compaction base cases.) A sufficiently large batch of compost material was prepared for three reactors each at two levels of compaction, 10% and 20%. This was done by use of a specific volumetric BA/S ratio, the densities of the chips and sludge, and addition of the appropriate percentages of additional mass for each test. 10% compaction was achieved by simply filling the reactor to the 250 ml level, noting the mass, adding 10% more, and compression with the screen and spacer; 20% compaction was achieved in a similar fashion. Tests were done at two different bulking agent to sludge ratios, 1.1/1 and 3.3/1, volumetrically.

TABLE 6

MAKE UP OF COMPACTION TESTS
(in grams)

Test #	BA/S Ratio (vol/vol)	Compac-tion	Total Sludge (wwt)	Chips (wwt)[b]	% HC (dwt)	Added Water	Overall MC
361p42[a]	1.1/1	0%	119.04	17.28	26%	0	30%
361p50	1.1/1	10%	184.50	26.78	26%	13.00	30%
361p51	1.1/1	20%	199.99	29.03	26%	16.00	30%
361p40[a]	3.3/1	0%	40.00	17.28	20%	0	41%
361p48	3.3/1	10%	88.00	38.02	20%	28.00	40%
361p49	3.3/1	20%	96.00	41.47	20%	32.60	40%

[a] p40 and 42 were run as tests in the BA/S ratio series.
[b] Wood chips averaged 7% moisture content.

Optimal Conditions Tests

Following the completion and interpretation of all the previous parameter tests, optimal ranges or values from each were incorporated in one test to determine if the combined effects were cumulative. For reasons detailed in Chapter 5, simple comparison of constant respiration rates was the best method for determining optimums. Permutations from the optimum test were made by individually varying each of the parameter values away from its optimum. This method resulted in seven different combinations for tests. The anticipation of this approach was to reveal whether the combined set of optimal conditions was truly a global optimum.

Due to time and space limitations, the number of replicates for each test was reduced to two. One respirometer was set to 40°C, the temperature optimum, and the other at 25°C. The parameter values selected for the optimal conditions test and its permutations are shown in Table 7.

TABLE 7

OPTIMAL TEST COMBINATIONS

Parameter	Optimal Conditions Test	Permuted Parameter[c]					
		% HC	BA/S Ratio	% CC Chip	Nutr. Conc.	Temp.	Comp.
% HC (dwt)	26%	17%	26%	26%	26%	26%	26%
BA/S Ratio[a]	1.1/1	1.1/1	3.3/1	1.1/1	1.1/1	1.1/1	1.1/1
% of Chip CC	33%	33%	33%	83%	33%	33%	33%
Nutrient Conc[b]	0.5 M	0.5 M	0.5 M	0.5 M	1.0 M	0.5 M	0.5 M
Temperature	40 C	40 C	40 C	40 C	40 C	25 C	40 C
Compaction	0%	0%	0%	0%	0%	0%	20%

[a] vol/vol basis
[b] as ammonium concentration
[c] underlined values indicate the change from the best combination

To lower the concentration of oil into the favorable range of 2 - 8% HC, 20 volumes of chips would have been required per volume of sludge. This seemed

impractical for field application, so HC concentration was subjugated to that which resulted from the selection of the BA/S ratio. The BA/S ratio chosen was 1.1/1 and resulted in a lowering of the dry weight HC concentration from 30% to 26%. The HC concentration permutation employed inert sludge solids material to further dilute the oil concentration to 15%, actually a step *towards* more favorable conditions.

Initially, the selection of the optimal value for *overall* moisture content was the basis for this selection. The result of this choice was a compost mixture that had the consistency of soup. It was then realized that 43% moisture content in a compost with a volumetric BA/S ratio of 13.2/1 was vastly different than 43% moisture content for a BA/S ratio of 1.1/1. The water content optimum was then re-defined in terms of wood chip container capacity. The water amendment became a function of the wood chip amendment and was 33% of CC_C. The permuted case was 83% of CC_C.

Nutrient test results showed that the highest constant respiration rates could be achieved at concentrations of 0.4 moles/liter ammonium and above. The permutation was run at twice the concentration of the optimum test (1.0 versus 0.5 M ammonium).

No clear indication resulted from the compaction series of tests but common sense dictated that the less compacted a mixture was, the better oxygen would be able to penetrate the matrix. Zero compaction was used as the optimum and 20% as the permutation. A consideration not taken for this design was that of which BA/S ratio best resists the results of compaction. If this aspect had been a design consideration, a ratio of 3.3/1 would have been employed rather than 1.1/1.

Table 8 shows the make-up constituents for the optimal conditions series of tests. The mass of sludge is held constant throughout the series, except for the compaction permutation, wherein a larger mass is necessary for the compaction vessel. In this series the "best combination" test is the base condition case. The resulting base condition dry weight hydrocarbon concentration is 26%. Additions of either chips and/or inert material serve to dilute the concentration. The physical quantity of added nutrient solution varies

broadly due to large variance in added water. More nutrient must be added to the larger volumes of water to achieve a constant concentration. More water also was necessary to fulfill the container capacity requirement for increased masses of chips.

TABLE 8

MAKE-UP OF OPTIMAL CONDITIONS TESTS
(in grams)

Conditions of Test Constant Rate BOD Based:	Test #	BA/S (vol. basis)	Sludge Mass	Chips Mass (dwt)[c]	HC Conc. (dwt)	Nutr. Soln.[a]	Make-up Water	Overall Moisture Content
Best Combination	361p57	1.1	59.02	8.00	26%	4.21	4.29	27%
15% HC w.r.t. Inerts	361p62	1.1	59.02	14.00[d]	15%	8.20	19.68	29%
BA/S Ratio of 3.3/1	361p61	3.3	59.02	24.00	20%	7.73	17.05	34%
83% Chip CC Test	361p60	1.1	59.02	8.00	26%	6.67	14.03	36%
1.0 Molar Ammonia	361p59	1.1	59.02	8.00	26%	8.42	0.69	26%
25°C Temperature	361p63	1.1	59.02	8.00	26%	4.21	4.29	27%
20% Compaction	361p58	1.1	194.77[b]	26.40	26%	13.89	14.15	27%
Specific Growth Rate Based:								
Optimum (35°C)	361p67	2.0	59.02	14.00	23%	2.74	32.05	43%
Permutation (25°C)	361p65	2.0	59.02	14.00	23%	2.74	32.05	43%

[a] Nutrient solution was 2.41 Molar w.r.t. ammonia and had approximate density of 1.17 gm/ml.
[b] Compaction test had to have 250 ml + 20% volume.
[c] chip moisture content was 10%.
[d] Added inerts test required added bulking agent to maintain 1.1/1 ratio.

Two separate, additional tests were run and were based upon the conditions which could be identified by specific growth rate analysis to be optimal for rapid microbial growth. These conditions were; a water content of 83% of CC_{chips}, an Evans' nutrient concentration of 0.14 M ammonium, a temperature of 35°C, no compaction, and an arbitrarily chosen BA/S volume ratio of 2:1 (resulting in an HC concentration of 23%). One permutation was run at a temperature of 25°C.

Extraction of Hydrocarbons

Three finished tests were selected for extraction of their hydrocarbons from among the optimal conditions tests. The basis for selection was the three which attained the highest cumulative level of BOD (mg O_2/gm VS). A control sample was prepared as would normally be done for duplicate tests in this series. It was then frozen until the time of extraction. At the conclusion of their respirometer runs, the selected tests were frozen until the time they could be extracted.

Extraction was performed in Tecator brand Soxtec HT2 1045 extraction units using a Soxlhet extraction process with methylene chloride. Frozen compost samples were broken up with an implement and transferred to an 800 ml plastic mix container. The reactor was twice rinsed with a minimal amount of methylene chloride, the rinsate being added to the mix container. Diatomaceous earth (DE) was added in an approximate one to one volume ratio to the compost and mixed well. The resulting volume of compost and DE mixture from one reactor required the use of three 30 mm X 65 mm extraction thimbles. The mix container was twice rinsed with methylene chloride, the rinsate being added into the top of the thimbles.

Thimbles were lowered into glass extraction cups containing 50 ml of methylene chloride and were boiled at a temperature of 130°C for 1 hour. After boiling, thimbles were raised and rinsed for two hours. At the conclusion of rinsing, the return valve was closed and solvent was boiled off for one half hour before the temperature was reduced to 100°C for half an hour, then lastly to 60°C for another half hour. This gradual reduction in temperature ends up below the boiling point of hexane (69°C) and was done to avoid boiling off lighter hydrocarbons. Extraction cups were removed and air dried under a hood for 12 hours, following which mass determinations were made.

CHAPTER IV

COMPOST BIODEGRADABILITY TESTING

Means of Evaluation and Comparison Among Tests in a Series

A typical oxygen uptake curve consists of several distinct intervals which can be named according to the type of microbial activity which is dominant through them. Figure 6 shows *ideal* cumulative oxygen uptake and instantaneous rate curves. A lag period generally precedes the beginning of microbial activity. It represents a time of acclimatization. In the case of the sludge employed in this study, which had microbial populations on the order of 100 to 1,000 per gram, it may represent uptake below the limit of pressure loss detection in the respirometer. The exponential phase represents rapid microbial growth taking place in the presence of an abundance of substrate, nutrients, oxygen, space, and other factors. The rate of exponential growth can be limited by excessive substrate or nutrient concentrations, or by other factors such as the temperature or moisture content of the compost. Exponential growth cannot proceed indefinitely. Eventually, something in the environment will become limiting. An example would be the finite area of exposed hydrocarbons which would limit both direct microbial contact with the hydrocarbons and its rate of solubilization into water. Another example is restricted permeability of the compost to air which would limit the availability of oxygen to the microbes. An imposed growth limitation commonly results in a period of constant respiration. Ideally, the rate limitation which terminates growth and results in constant respiration is caused by the parameter under investigation. Constant respiration can be ultimately terminated by either oxygen starvation due to KOH exhaustion or poisoning by

metabolic products, which results in the decay phase. Culture death due to anoxia can be suspected when the total BOD exerted over the span of a test exceeds approximately three times the stoichiometric amount allowed by the available KOH, or approximately 0.75 gm O_2/gm KOH (see Appendix A for details).

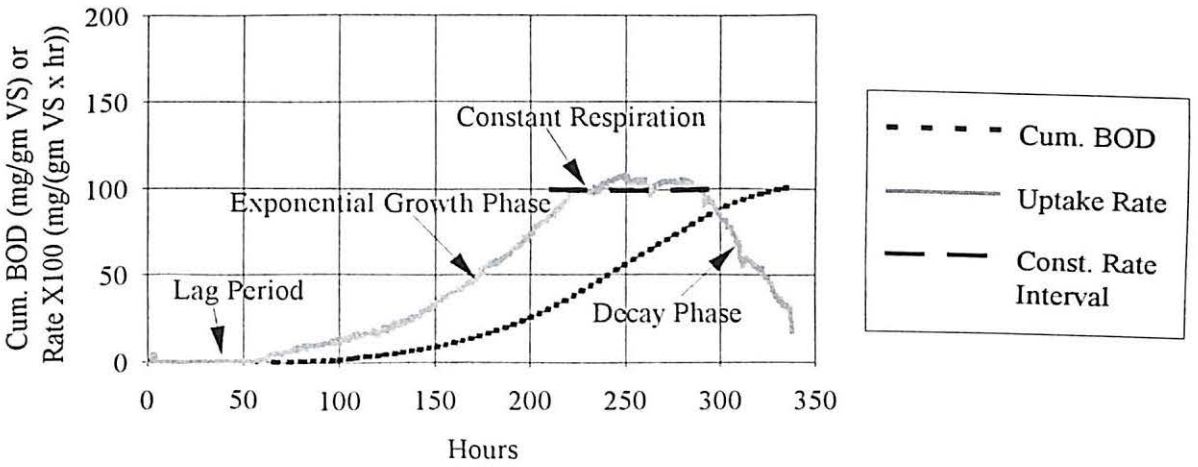

Figure 6. "Ideal" Plot of Oxygen Uptake Data

Several other explanations are possible for the onset of the decay phase, though during short term respirometry, some are not likely to result. In tests of compost with high concentrations of hydrocarbons, it is unlikely that the carbon food source would become exhausted. It is not likely that all species of a heterogeneous microbial population would exhaust their preferred hydrocarbon substrates simultaneously. When an oxygen uptake curve breaks over from exponential growth into a plateau of constant rate respiration, the population as a whole has been affected by a growth limitation. Though nutrients could become growth limiting in their availability once they are all incorporated into biomass, it is not possible for them to become exhausted since they are confined to the system and are always available through endogenous respiration.

Figure 7 shows an example of an uptake rate curve which drops off rapidly from its peak rate. There is no obvious subsequent interval of constant rate respiration. A possible explanation is that oxygen starvation or poisoning of the culture has occurred,

thus precluding the development of a constant rate plateau. Since starvation or poisoning cannot be positively concluded as the cause, uptake curves of this nature were analyzed for a constant rate across a 50 hour interval straddling the peak rate. The result is a conservative value for constant rate BOD compared to what might result in the absence of a rapid decay phase. In other words, a plateau may have been initiated beginning at the peak rate (212 mg O_2/gm VS) but for the influence of something fatal to the culture.

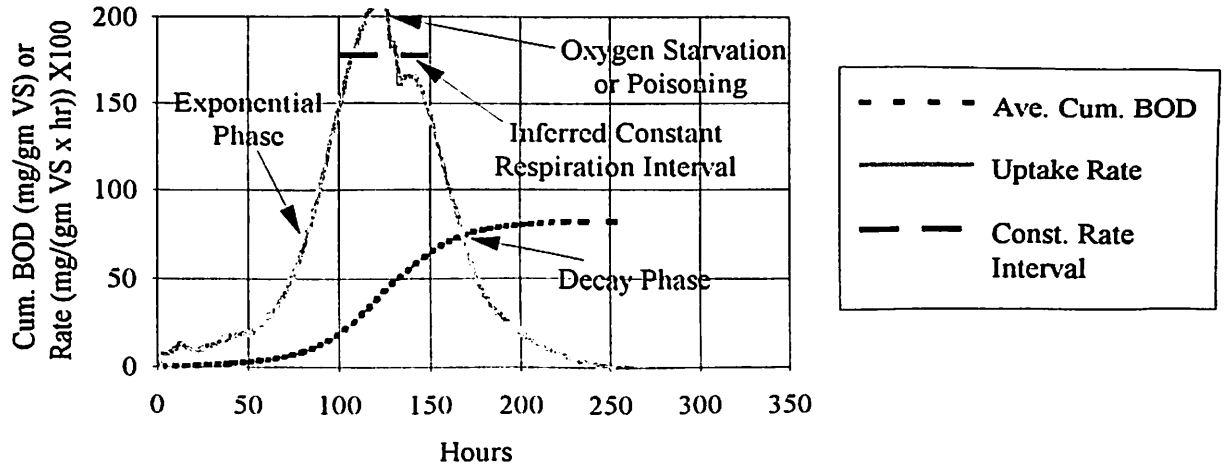

Figure 7. Example of Possible Oxygen Starved or Poisoned Culture

Constant Rate BOD

Constant uptake rate respiration (constant rate BOD) generally results when exponential growth expires as was shown in Figure 6. The level to which this rate rises is a function of the specific conditions of the test. The constant rate level of respiration is determined by inspection of a plot of a test's "instantaneous rate" of oxygen uptake. Instantaneous rate is determined simply as the difference of each pair of adjacent points on the cumulative uptake record, as shown in equation 8. This is accomplished quickly in a spreadsheet and when the values are multiplied by 100, they can be plotted on the same scale as the cumulative oxygen uptake data. The resulting curve is a first derivative that indicates the rate of change at any point in the cumulative uptake curve. Most commonly

the constant rate interval is chosen following the end of the exponential growth phase and before the beginning of the decay phase. Sometimes it is not clear, as in the case shown in Figure 8, which interval best represents constant rate uptake. In this case, an interval was chosen across both plateaus, whose average rate was deemed best representative of "constant rate". The difference between the two plateaus is evident as an inflection in the cumulative BOD curve. This appearance is common to tests in this experiment which greatly exceed a "minimal salts" concentration of nutrients. It is not understood why this is so, but may represent the separate growth cycles of two species dominating the microbial community.

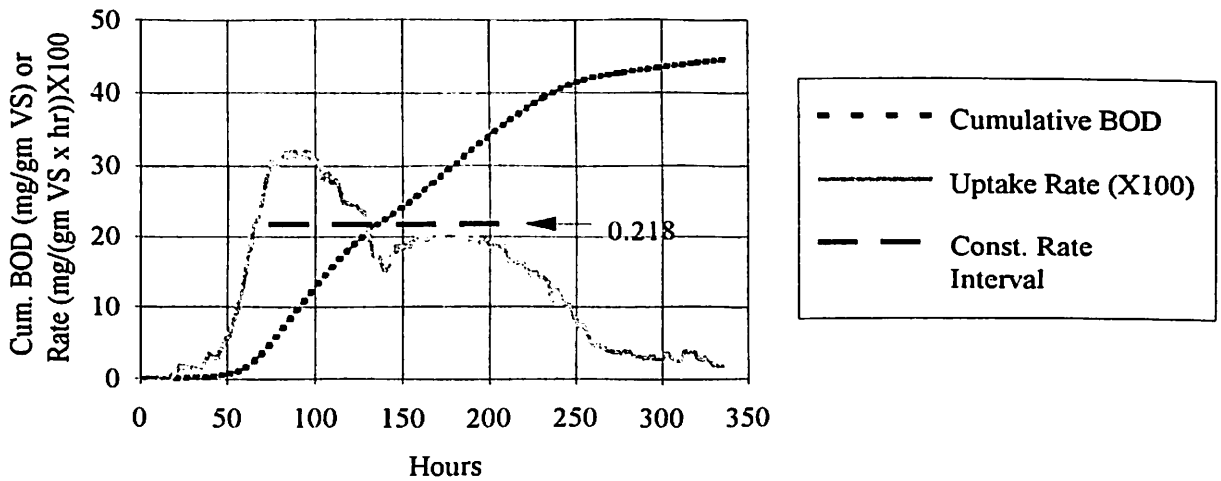

Figure 8. Average Constant Rate BOD Interval Across Plateaus

Another common appearance among oxygen uptake rate curves is one shown in Figure 9. It is characterized by a very rapid escalation in uptake rate to a peak, followed by equally rapid decline to a plateau of constant respiration.

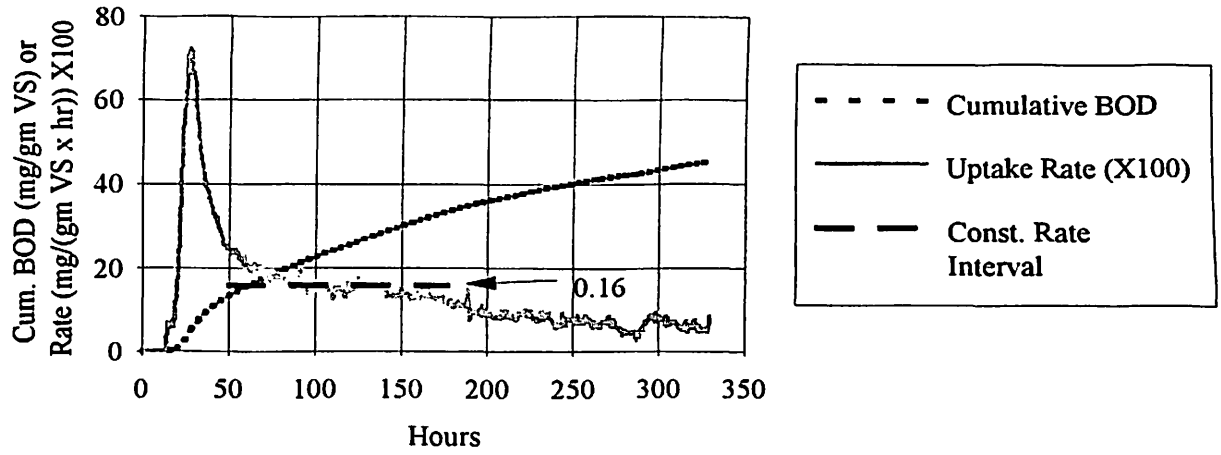

Figure 9. Rapid Rate Escalation and Decline to Plateau

Another pattern of oxygen uptake prevailed in certain tests with low levels of microbial activity. These low uptake rates always coincided with tests devoid of a nutrient supplement. The pattern was in cycles consisting of spurts of oxygen deliveries followed by extended periods without a delivery. These cycles were common to tests with very low oxygen demands and which were run in a respirometer later discovered to have leaks in its piping. The leaks were not to the reactors but were in connections to the reference chambers. Leaking occurred during periods of barometric change, causing the computer to deliver excess oxygen, beyond BOD, to reactors when atmospheric pressure increased. A decrease in barometric pressure would result in extended periods without oxygen deliveries, below BOD. The ending cumulative BOD was believed to be minimally affected. Therefore, for this type of uptake data, a constant rate was taken as the slope of a trend line fitted by method of least squares to the *cumulative BOD* curve. Figure 10 is an example of a test with episodic oxygen delivery.

Scaling for x and y axes for a series of tests was determined by the maximum range of values experienced within that series. Later tests which commonly employed nutrient supplements ran for 336 hours and experienced BOD of nearly 100 mg oxygen/gm volatile solids (VS). Some tests experienced such low oxygen consumption that they would not

be discernible on a chart so scaled. The test portrayed in Figure 10 barely exceeded 1 mg oxygen/gm VS.

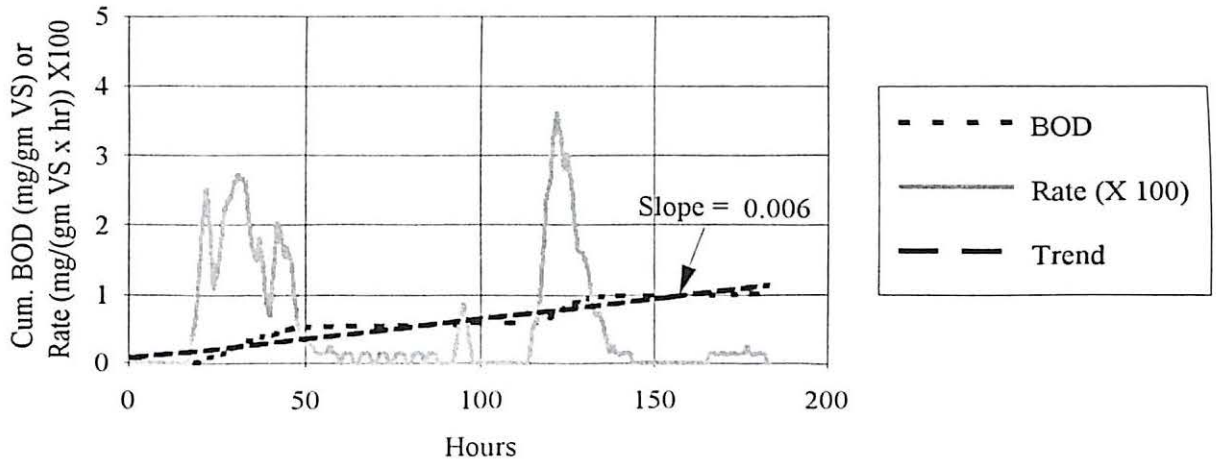

Figure 10. Erratic Uptake Data from Marginally Active Tests

Specific Growth Rate Estimation

Another basis for quantifying and comparing activities among tests is by estimation of specific growth rates. Specific growth rate (μ) is the rate of change in a population per unit of existing biomass over time, as shown in Equation 1. Higher values of μ reflect more rapid growth and viability of the culture for the given set of conditions. Among a set of tests which varies a single parameter, the highest specific growth rate indicates the value of the parameter most favorable for rapid growth.

A graphical technique for determining μ calls for plotting instantaneous oxygen uptake rate as a function of cumulative uptake (Figure 11). The early portion of the plot shows a linear interval which begins at zero and represents the exponential growth phase of the culture. The slope of a trend line fitted by method of least squares to this interval is an estimate of the specific growth rate of the culture. This technique is justified through Equation 12 in Chapter II and demonstrated in the following Equation 15, where the last expression is one which determines the slope of the trend line.

$$\mu = \frac{dX/dt}{X} = \frac{d(O_u/dt)}{dO_u} = \frac{\frac{dO_{u_2}}{dt} - \frac{dO_{u_1}}{dt}}{O_{u_2} - O_{u_1}} \tag{15}$$

Figure 11 shows an ideal example of uptake rate versus cumulative oxygen uptake, the fitted trend line, and the slope estimate for μ.

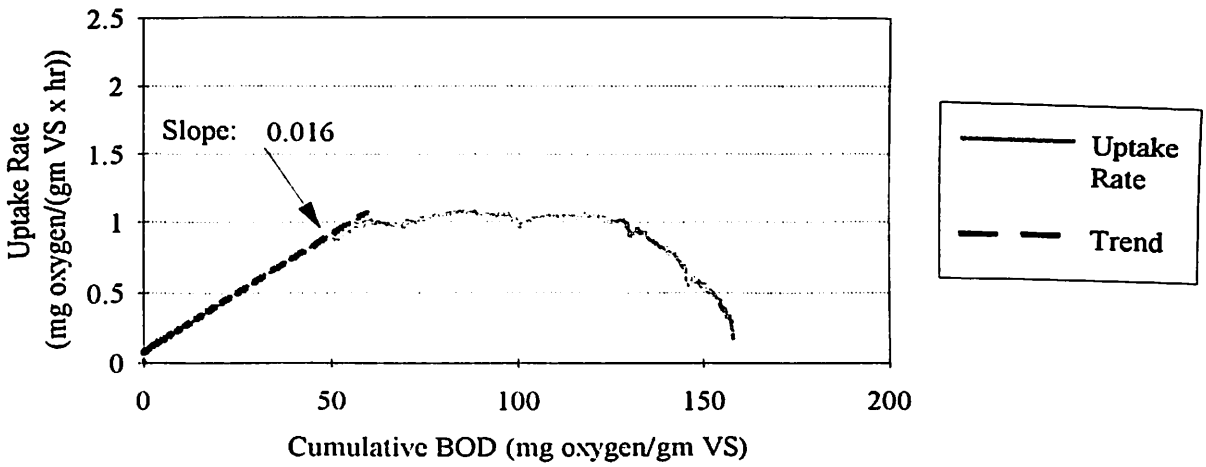

Figure 11. "Ideal" Plot of Rate Versus Cumulative Uptake

Sometimes the uptake rate plot levels gradually to a constant rate, as seen in Figure 12. It is useful in this case to also examine the test's corresponding average cumulative uptake and rate plot (Figure 7) for help in selecting the appropriate interval for trend line analysis. In this case, the interval selected for a trend line fit does not include the peak uptake rates incurred in the test. Initial exponential growth ends around 50 mg/gm VS cumulative uptake. The termination of exponential growth is not always a clear-cut event.

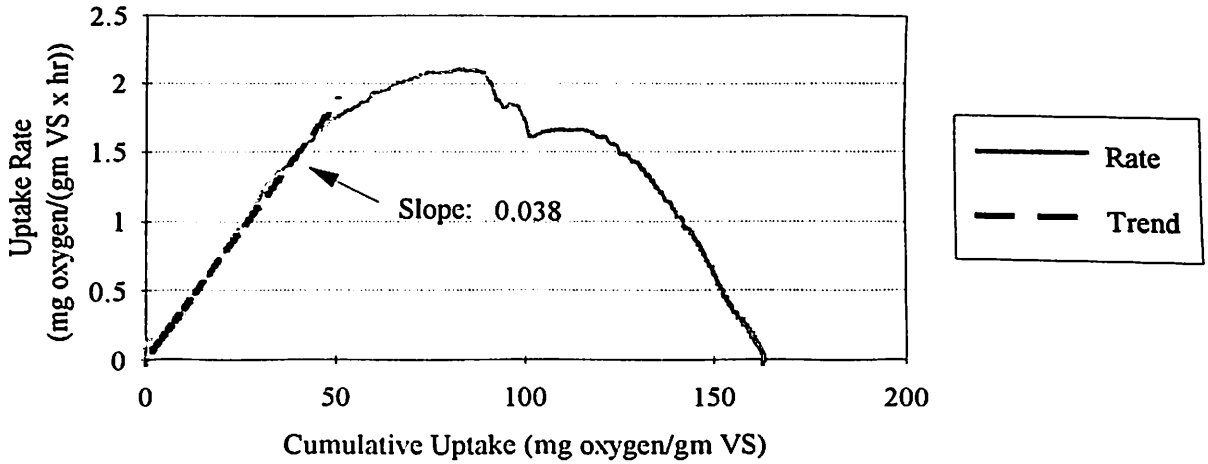

Figure 12. Gradual Termination of Exponential Growth

Total BOD can be so low that uptake data do not extend far from zero (Figure 13). In some extreme cases, particularly those tests lacking any nutrient amendment, the erratic character of the plot is not suitable for making a trend line fit or slope estimate. In those cases specific growth rate is not estimated.

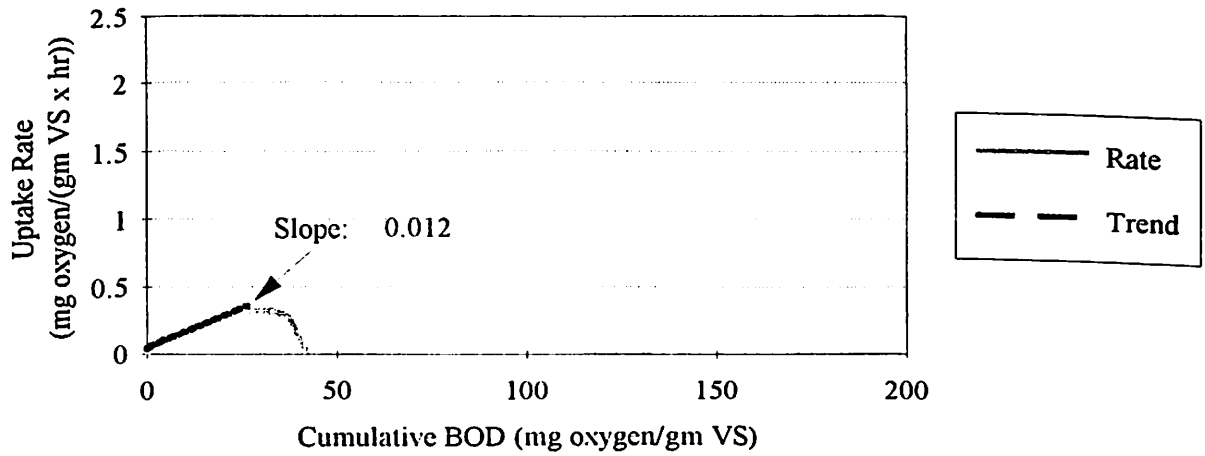

Figure 13. Rate Versus Cumulative Data for Low Uptake

CHAPTER V

RESULTS AND DISCUSSION

General Discussion

Results from all series of tests consisted first of raw oxygen uptake data recorded on an hourly basis in the form of accumulative values. Uptake was recorded as mass of oxygen delivered per unit mass volatile solids or mass total solids in the sample. Tests in all series, except the optimal series, were run as triplicates.

Every set of tests had three types of graphs created from it's uptake data. The first graph is a composite of the individual cumulative oxygen uptake curves from each reactor in the set. This type of graph provides a visual indication of the variability within a set. A second type of graph displays the average cumulative BOD of the three series of data from one set along with their average instantaneous rate of uptake (X100). This graph also shows the interval selected for constant respiration rate and the value of that average rate. The third type of graph depicts average oxygen uptake rate plotted against average cumulative BOD. This graph shows a least squares fitted trend line for the early linear segment of the rate curve. The slope of the trend line is representative of the specific growth rate (μ) of the microbial culture.

This research was designed without regard for the special requirements necessary for obtaining valid specific growth rate data. One requirement was that all factors contributing to microbial growth be present in abundance except for the one whose limitation is under investigation. Initially, it was not known that the low concentrations of nutrients in Evan's media could be growth limiting. Consequently, specific growth rate

results for some series of tests were from conditions less than ideal. Three experimental series which did meet the requirements for valid specific growth rate determinations were the nutrient, part of the temperature, and the optimal conditions experiments.

Hydrocarbon Concentration Test Results

A summary of constant respiration and specific growth rates is shown in Table 9. Two series were run with hydrocarbon concentrations of 0%, 2.5%, and 5.0% common to both sets. The resulting uptake curves on the whole were erratic. Since nutrients were present only at the low concentrations available in standard Evans' media, it is suspected that microbial activity suffered as a consequence. The maximum deviations among replicate tests from their average are shown in absolute terms and as a percentage of their average constant rate BOD. These maximum deviations can be taken as a conservative estimate of data variability.

TABLE 9

HYDROCARBON CONCENTRATION TEST RESULTS

	Test #	HC Conc. (dwt)	Constant Rate BOD		Specific Growth Rate
			Average[a]	Max. Dev.[a]	
Series 1	337p72	0.0%	0.011	0.006 (60%)	0.009
	337p82	2.5%	0.082	0.018 (22%)	0.046
	337p73	5.0%	0.049	0.025 (50%)	0.020
	337p83	7.5%	0.096	0.020 (20%)	0.033
	337p70	10%	0.045	0.012 (26%)	0.021
	337p74	15%	0.031	0.005 (17%)	0.047
	337p71	20%	0.025	0.002 (7%)	0.046
Series 2	337p104	0.0%	0.019	0.002 (10%)	0.000
	337p94	0.5%	0.053	0.004 (8%)	0.090
	337p93	1.0%	0.036	0.006 (16%)	0.162
	337p92	2.5%	0.078	0.003 (4%)	0.066
	337p91	5.0%	0.070	0.007 (10%)	0.049

[a] mg oxygen/(gm VS x hr)

Constant Rate Analysis

The summary graph depicting how average constant rate respiration varied with hydrocarbon concentration is shown in Figure 14. Individual rates between the two series at the repeated concentrations of 0% and 2.5% HC were similar. One anomalously low rate at 5.0% concentration lowered the series 1 average significantly, whereas the five other individual rates at that concentration were all close as a group. In series 1, variability gradually attenuated in the direction of increasing HC amendment and concentration (>10%). Overall, series 2 exhibited less variability than series 1. There is an unexplainable decrease in rates from the 0.5% to the 1.0% HC tests, both of which are in the same series.

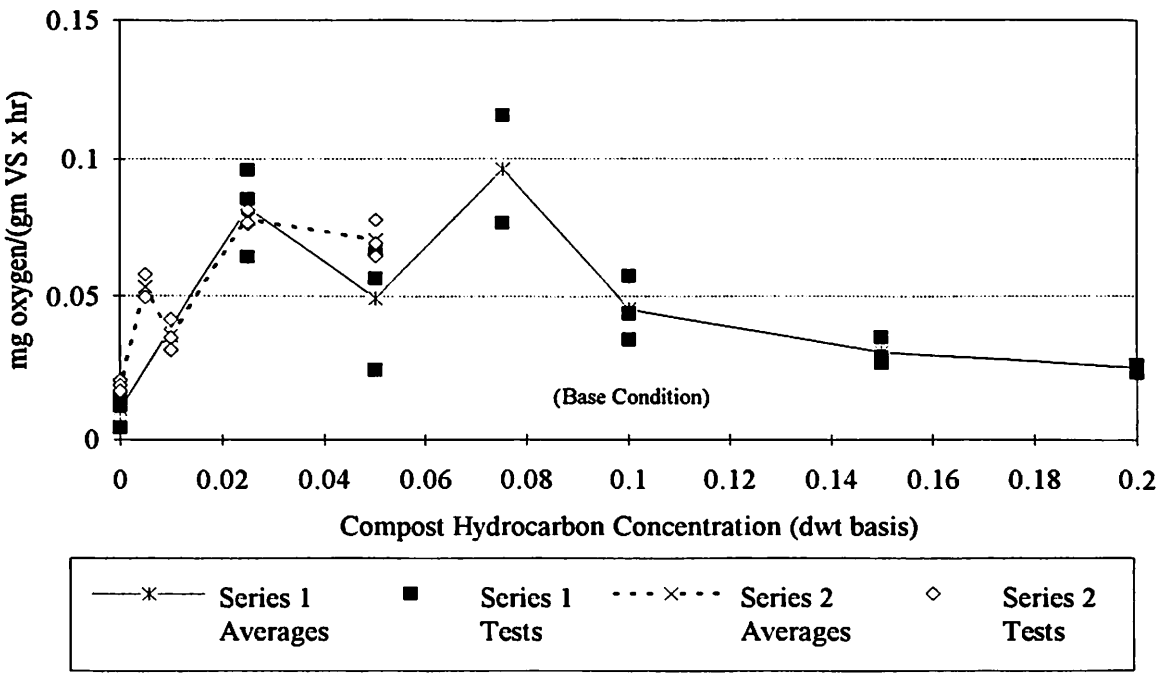

Figure 14. Average Constant BOD Rates vs. HC Percentage

There are many ways to explain variability among individual tests and between series. Heterogeneities in the sludge may introduce variations in hydrocarbon substrate or

microbial population. Populations may change with shelf time and vary between storage containers. Inconsistencies in the size distribution of wood chips may result in different surface areas among tests. Through the course of filling reactors, and despite efforts to assure uniformity, invariably the last reactor filled received the "dregs" of a mixture. This last material generally was of finer size and contained extra liquid if there was much free water in the batch mixture. Though an uncommon occurence, the accidental spilling of KOH solution within a reactor can toxify a portion of its contents.

The 0% concentration tests averaged 0.015 mg oxygen/(gm VS x hr). This value can also be taken to represent the background level of activity on the chips themselves. The highest average rates occurred in tests with between 2% and 8% HC concentration. The highest uptake rate, at 7.5% HC, was 6.4 times higher than the rate on wood chips alone.

An interesting point about the constant rate results is that to achieve a HC concentration within the optimal range of 2% to 8% (dwt), 24% TPH sludge would have to be diluted considerably with bulking agent. An example is one of the experimental tests itself, #337p70, the "base condition" for this series. The volume of chips necessary to reduce the 24% TPH sludge to 10% (dwt compost) was 13.2 times the volume of the sludge itself. High volume bulking agent dilution of sludge may not be a feasible option for field operations, so an alternative method of sludge dilution may be considered. Such an alternative might be dilution by means of added low level HC contaminated soil in place of a portion of the bulking agent. The effect could be the same as adding bulking agent by lowering the dry weight concentration of hydrocarbons into a favorable range. This argument can be supported by observing that, with a decreasing ratio of sludge to total inert material below 10% HC, an increase in constant BOD results. This approach was used as a permutation within the "optimal conditions" series of tests.

Specific Rate Analysis

Specific growth rates determined graphically from oxygen uptake data show the highest growth rates occur at 0.5% and 1% hydrocarbons concentrations (Figure 15). For most tests, particularly those of series 2, the uptake data were of good quality and amenable to specific growth rate estimation. Ammonium concentration through the HC series was about 0.014 M, well below the range fostering the highest growth rates in the nutrient addition series of tests. Despite this, μ's for both 0.5% and 1.0% HC concentrations were higher than the highest rate observed in the later nutrient addition experiment (0.090 $hr^{-1}$and 0.0162 hr^{-1} versus 0.068 hr^{-1}). The nutrient experiment employed a 10% HC concentration and experienced highest growth rates at 0.11 M to 0.17 M ammonium, a C:N ratio of from 32:1 to 47:1. The highest rate in this HC concentration series was at 1.0% HC and 0.014 M ammonium, a C:N ratio of 38:1. This suggests the possibility that there may be a hydrocarbon/nutrient ratio effect wherein highest growth rates are associated with a certain range of ratios. Otherwise, it can be concluded that at low nutrient levels, hydrocarbon concentrations have little affect on initial rates of growth until they drop below 2.5%.

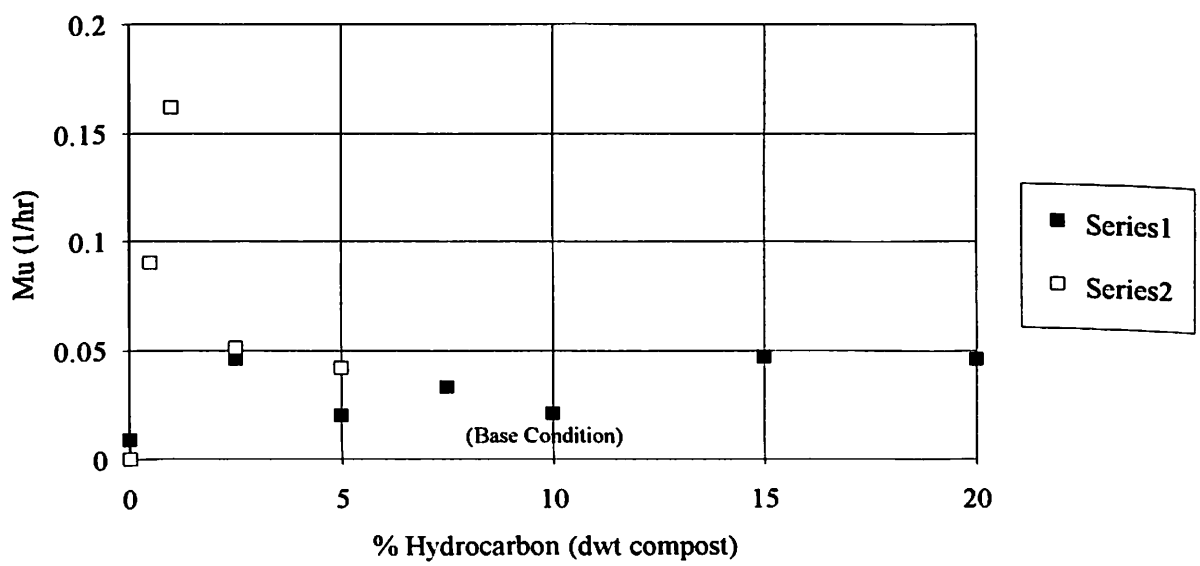

Figure 15. Specific Growth Rates (μ) for Various Hydrocarbon Concentrations

Bulking Agent/Sludge Ratio Test Results

Table 10 presents the rate results for this series of tests. In the previous series of hydrocarbon concentration tests, a base condition had been chosen at a bulking agent to sludge ratio of 2:1, on a dry mass basis. Those proportions resulted in a 13.2 to 1 ratio on the basis of volume. Series 2 investigated very high BA/S ratios and extended coverage to the extreme of 191 volumes of chips per volume sludge. Series 3 investigated very low BA/S ratios.

TABLE 10

BULKING AGENT/SLUDGE RATIO TEST RESULTS

	Test #	BA/S Ratio (vol/vol)	% HC (dwt)	Constant Rate BOD Average mg/(gm VS x hr)	Constant Rate BOD Max. Dev. mg/(gm VS x hr)	μ (1/hr)
Series 1	337p97	32.9	5.0	0.047	0.007 (14%)	0.025
	337p98	13.2	10.0	0.062	0.005 (8%)	0.049
	337p99	6.6	15.0	0.056	0.013 (24%)	0.053
	337p100	3.3	20.0	0.045	0.007 (15%)	0.080
	337p102	0.0	30.0	0.012	0.0003 (3%)	0.027
Series 2	361p22	191	1.0	0.050	0.002 (4%)	0.009
	361p21	72	2.5	0.047	0.005 (10%)	0.012
	361p20	33	5.0	0.059	0.003 (5%)	0.007
	361p19	20	7.5	0.053	0.011 (20%)	0.006
Series 3	361p40	3.3	20.0	0.061	0.011 (18%)	0.011
	361p41	2.2	22.6	0.062	0.006 (9%)	0.033
	361p42	1.1	25.7	0.061	0.011 (17%)	0.034
	361p44	0.8	26.6	0.042	0.002 (6%)	0.022
	361p45	0.6	27.7	0.036	0.007 (20%)	0.005

Constant Rate Analysis

The results show the quality of the data to be only fair within each separate series, and between series, the repeatability was poor. The average rates of tests repeated in

different series (at 3.3/1 and 33/1 BA/S ratios) showed discrepancies of up to 24%. Their variability can be attributed to the same factors presented in the discussion of HC concentration constant rate results (different containers of sludge, etc.). Figure 16 shows the average and individual constant BOD rate results.

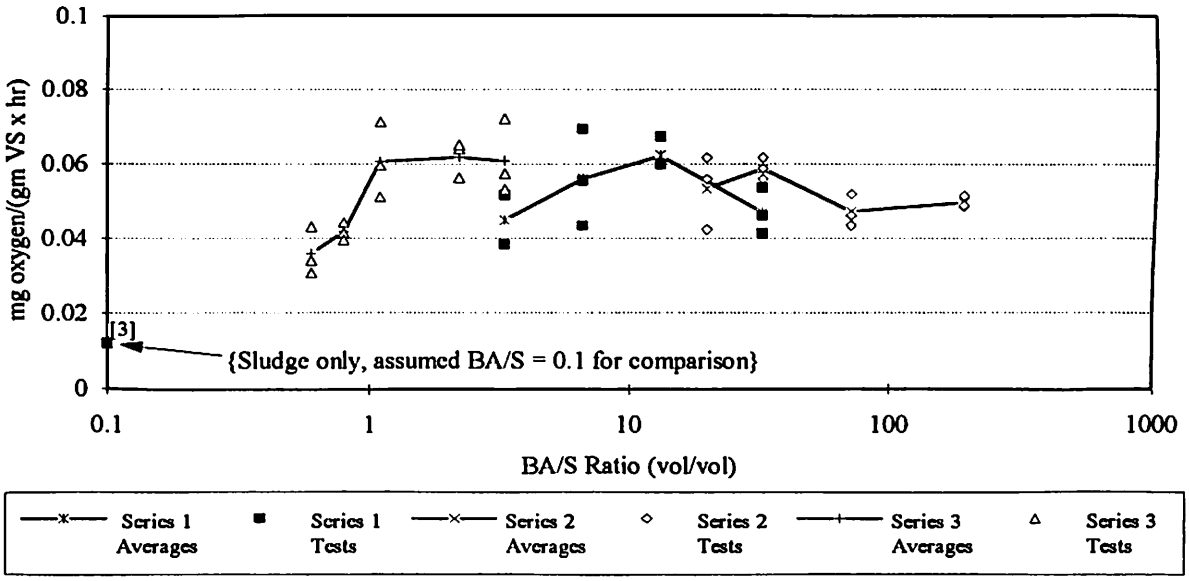

Figure 16. Constant BOD Rates versus Bulking Agent/Sludge Ratio

Constant BOD rates fell off rapidly below the ratio of 1.1/1 in series 3. Those low rates were only nominally lower than some low rates of series 1, but were still substantially above the rates for the "sludge only" triplicates (2.4X greater). This indicates that even low bulking agent/sludge ratios, down to 0.6/1, are beneficial to microbial activity. Above a 1/1 ratio the rates are widely distributed in the range of 0.04 to 0.07 mg oxygen/(gm VS x hr). The range gradually attenuates with increased bulking agent proportion. Overall, these results suggest that there is some critical BA/S ratio, in this case about 1/1, above which microbial rates are consistently higher. It is also apparent that there is no advantage in exceeding that critical ratio, unless it is for other considerations (i.e. compaction, water holding capacity).

It should be noted that what is really being varied in this series is the accessibility of the sludge microorganisms to air (permeability). This results from increases in the exposed surface area of sludge from addition of ever greater proportions of wood chips. Soils or sludges of different consistency and texture may require more or less bulking agent to achieve the same results, so the indication that a BA/S of 1/1 or greater is most favorable for activity should be regarded as unique to this particular sludge and bulking agent.

Specific Rate Analysis

The erratic distribution of the rates shown in Figure 17 indicate the BA/S oxygen uptake data did not lend itself well to specific growth rate interpretation. Series 3 in particular was characterized by rapidly fluctuating uptake rates for which it was difficult to assess the period of exponential increase. The erratic rate results are speculated to be due to: 1) low nutrient concentrations in standard Evans' media (0.014 M ammonium), and 2) inconsistent nature of the substrate as a varying proportion of hydrocarbons to wood chips.

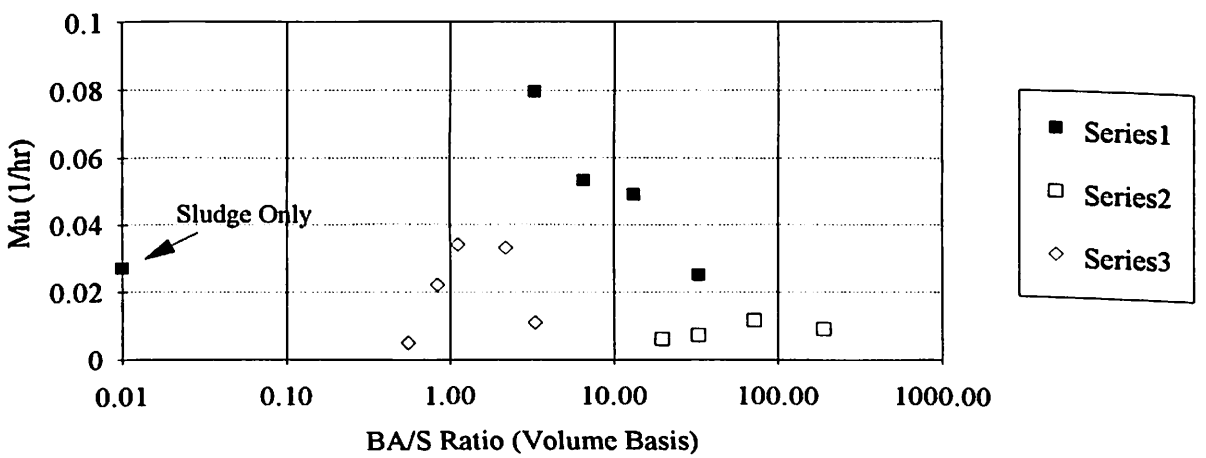

Figure 17. Specific Growth Rate Versus Bulking Agent/Sludge Ratio

Moisture Content Test Results

The overall quality of the data was considered fair. Several reactors exhibited fluctuations and periodic swings in uptake rate. In general, the higher the water content, the smoother the appearance of the uptake curves. Less erratic uptake could have been the result of a more continuous and uniform surficial fluid environment for microbial activity. Table 11 shows the average rate values from the series and maximum deviation of individual replicates from their average.

TABLE 11

MOISTURE CONTENT TEST RESULTS

% of Wood Chip CC	% of Compost CC	Moisture Content (%)	Constant Rate BOD		μ (1/hr)
			Average (mg/(gm VS x hr)	Max. Dev. (mg/(gm VS x hr)	
5	12	16	0.005	0.001 (22%)	undefined
33	46	43	0.073	0.016 (22%)	0.023
50	67	52	0.051	0.001 (3%)	0.022
67	88	58	0.044	0.009 (20%)	0.070
83	108	63	0.034	0.004 (12%)	0.182
100	129	67	0.029	0.002 (7%)	0.132

The design for the moisture content series was based upon independent variation of moisture levels as percentages of the water holding capacity of the wood chips. The sludge in its natural state was already water saturated and was measured to be slightly over its container capacity (CC_{sludge} = 0.22 gm H_2O/gm dry material), or nearly equivalent to its moisture content. The container capacity of the extracted chips was nearly three times their dry mass. Since the moisture content of the sludge was constant, it was decided to make the moisture content of the *compost mixture* a function of *wood chip* container capacity and vary CC_{chips} across the range of 0% to 100%.

Subsequent measurement of the container capacity of the resulting *compost mixture* revealed that it was not equal to the sum of the individual container capacities of the sludge and wood chips. The container capacity of the *compost* was 1.56 g H_2O/g dry material. Thus, the $CC_{compost}$ was 79% of the sum of CC_{chips} and CC_{sludge}. This was explained by sludge hydrocarbons being spread over the surface of the chips during mixing which preventing complete absorption of the added water. This also explained why there was free water in the bottom of the reactors run at chip container capacities above 79%. Figures 18 shows average constant BOD rates and individual test rates with respect to *chip* container capacity.

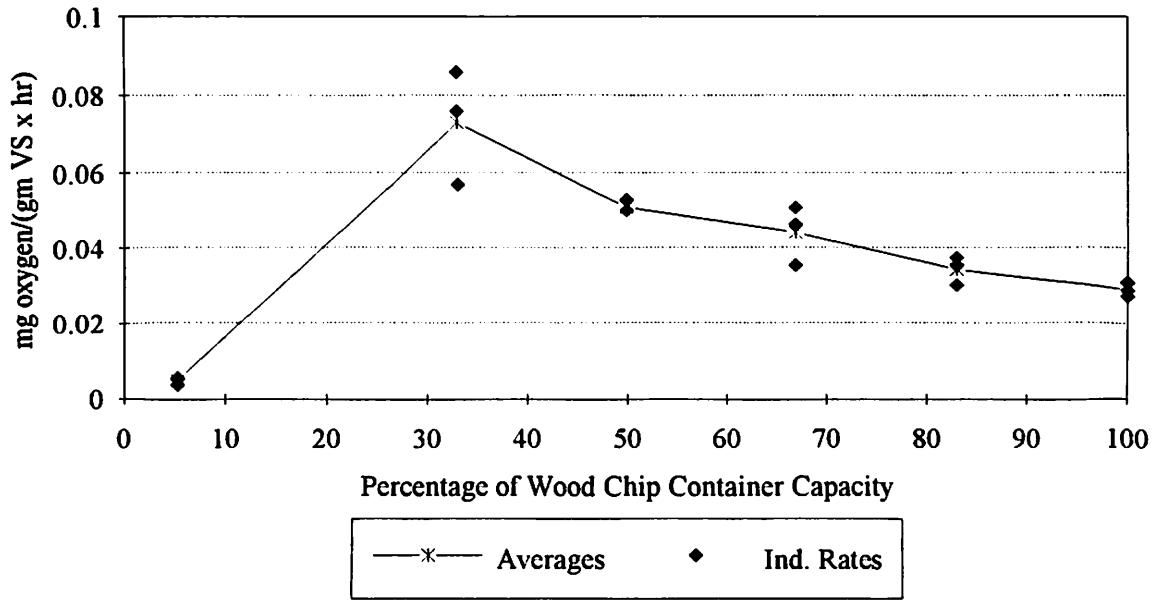

Figure 18. Constant BOD Rates vs. Wood Chip Container Capacities

Figure 19 shows the same rates with respect to *compost* container capacity. The range of free water observed in the reactors is indicated by the length of the arrow.

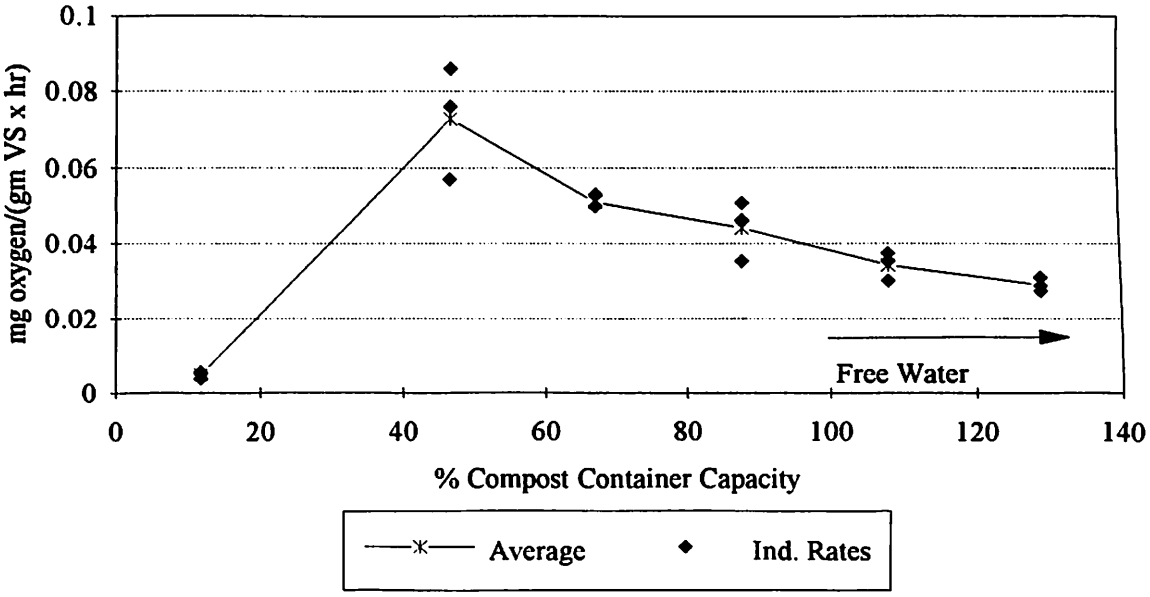

Figure 19. Constant BOD Rates vs. Compost Container Capacities

Constant Rate Analysis

Constant rate uptake is maximum at 46% of the compost container capacity (33% of chip CC). This water content also shows the greatest variability with a maximum deviation of 0.016 mg O_2/(gm VS x hr) from its average (or 22%). Between 12% and 46% $CC_{compost.}$, there apparently is a threshold moisture level above which microbial activity was greatly enhanced compared to that of the base condition (12% $CC_{compost}$), to which no water was added. Unfortunately, the range of these tests did not sufficiently cover that interval. None-the-less, the interval most conducive to microbial activity is the range from about 40% to 90% of $CC_{compost}$. Practically, any water content above 40% $CC_{compost}$ is preferable over the nearly dry state of the base condition test. In terms of CC_{chip}, the most favorable range is from about 30% to 70%.

Specific Rate Analysis

Specific growth rate increased dramatically above a water content of 88% of $CC_{compost}$ (Figure 20). This appears to have been associated with the occurrence of free water in reactors above that water content. A possible explanation is that the standing water provided an environment conducive to exponential growth (readily available nutrients, high rate of HC solubilization, etc.). The specific growth rates in these tests were the second highest (0.18/hr max) observed throughout the entire experiment, though they were of very short duration (8 to 10 hr). Within this series the maximum rate was nearly eight times greater than the lowest measurable rate. The level of activity was so low and erratic in the base condition test that μ could not be reliably determined.

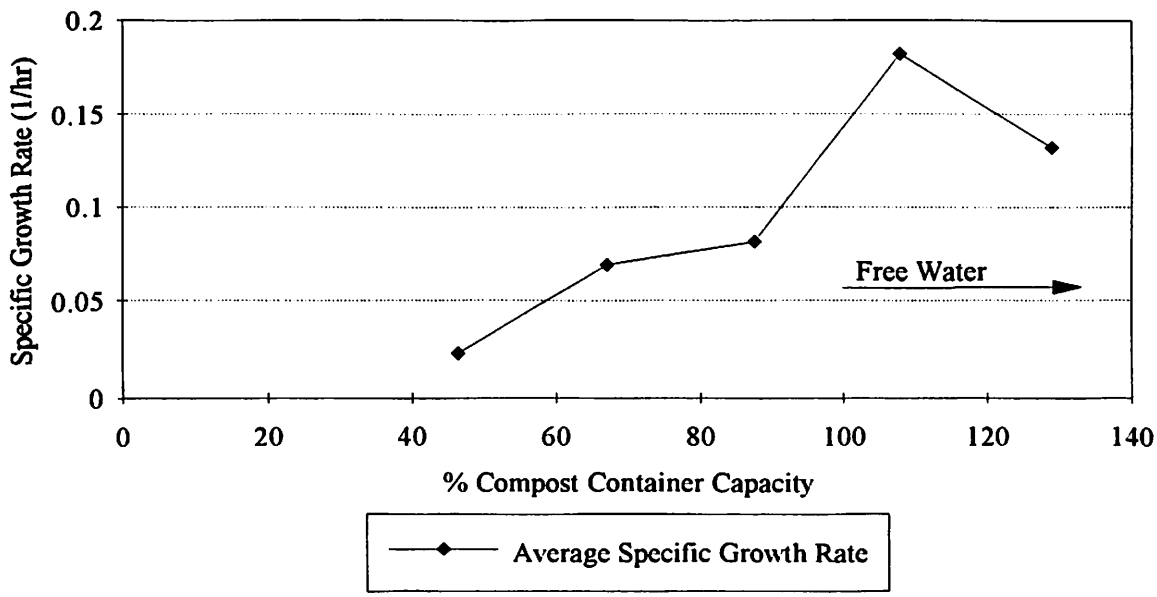

Figure 20. Specific Growth Rates vs. Compost Container Capacities

Nutrient Concentration Test Results

This series was unusual among the parameter tests because of the high rates that were associated with elevated concentrations of nutrients. Exponential growth was

sustained for longer periods of time than in any other tests (45 to 100 hours) to this point. Cumulative BODs all plateaued in the high range of 40 to 47 mg oxygen/gm VS, which is approximately 10% of the stoichiometric requirement for oxygen based on 3.48 gm O_2/gm HC. The indication is a possible across the board exhaustion of KOH. The levels of total BOD achieved ranged from 3.0 to 3.6 times the allowable oxygen due to KOH (see Appendix A for discussion on KOH capacity).

The rate results for this series follow in Table 12. Series 3 was a repeat of series 1, but was run for a longer period of time. The two higher concentration tests of series 1 terminated before a constant rate of oxygen uptake had been achieved, so no values appear for them. It was possible, however, to obtain specific growth rate estimates from the abbreviated data that were recorded.

TABLE 12

NUTRIENT ADDITION TEST RESULTS

	Test Number	Ammonium. Concentration (molc/L)	Constant BOD Rate		Specific Growth Rate (1/hr)
			Average (mg/(gm VS x hr))	Max. Dev. (mg/(gm VS x hr))	
Series 1:	337p87	0.000	0.006	0.000 (0%)	0.005
	337p88	0.225	0.261	0.040 (15%)	0.034
	337p89	0.450	n/a	n/a	0.025
	337p90	0.674	n/a	n/a	0.025
Series 2:	337p105	0.056	0.226	0.045 (20%)	0.012
	337p106	0.113	0.181	0.039 (22%)	0.068
	337p107	0.169	0.221	0.038 (17%)	0.067
	337p108	0.225	0.252	0.027 (11%)	0.050
Series 3:	361p08	0.000	0.010	0.001 (11%)	0.004
	361p09	0.225	0.356	0.105 (29%)	0.043
	361p10	0.450	0.444	0.025 (6%)	0.031
	361p11	0.674	0.535	0.290 (5%)	0.033

Constant Rate Analysis

Series 3 constant BOD rates followed a trend of increasing rates with increasing ammonium concentration. Series 2 results also followed an increasing trend, except for test 337p105, which on the whole showed anomalously high constant BOD rates. The variability among test replicates was low, except for 361p09, where one deviant replicate fell 29% below the test average. Interestly, it fell within the narrow range of deviation of two previous tests of the same nutrient concentration. Figure 21 is a summary graph showing average and individual constant BOD rates for each test set. Series 1 consists of only two sets of points, one at 0 M. NH_4^+ and the other at 0.225 M. NH_4^+. The three individual rates at 0 M. NH_4^+ for series 1 are hidden beneath another set of points from series 3. The repeatability of results from series 1 and 2 was good as shown by the close clustering of their individual rates at 0.225 M. NH_4^+. Series 3, however, defied this repeatability when two of its individual rates jumped significantly higher.

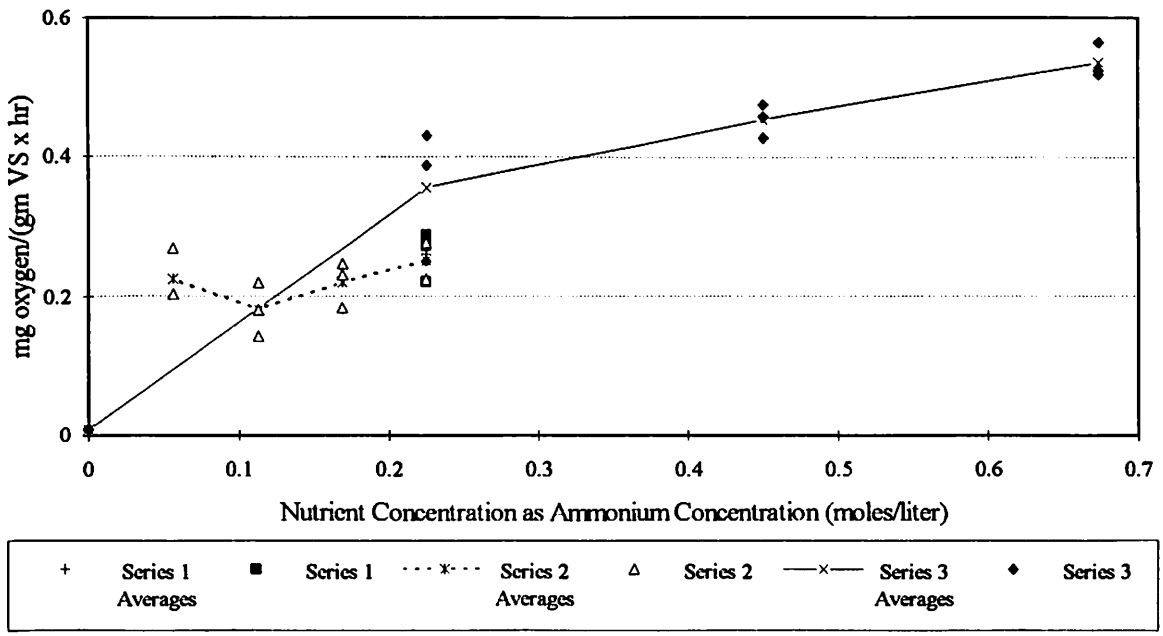

Figure 21. Constant BOD Rates for Various Nutrient Concentrations

It is evident that any Evans' formulation based nutrient amendment of at least 0.056 M ammonium is better than no nutrient amendment at all for improving respiration rates. Apparently, the higher the concentration, the higher the resulting respiration rate. Unfortunately, no tests with higher concentrations of nutrients were run in this nutrient experiment. However, one of the permutations within the later optimal series of tests was run at a concentration of 1.0 M ammonium and exhibited a greatly reduced rate compared to an similar test with 0.5 M ammonium concentration. That result suggests ammonium inhibition occurs at some concentration above 0.7 M. Inhibition of respiration rates with increasing ammonium concentration is documented and has been observed to reduce respiration rates to less than 20% of the maximum rate (Edwards, 1970). These results, as they stand, indicate the most favorable range for high constant respiration rates is approximately from 0.40 to 0.70 moles/liter ammonium.

Specific Rate Analysis

This series of tests, more so than any other to this point, provided the best conditions for the determination of specific growth rates. The addition of nutrients produced lengthy periods of exponential growth. Specific growth rate estimates for the various nutrient additions are shown in Figure 22.

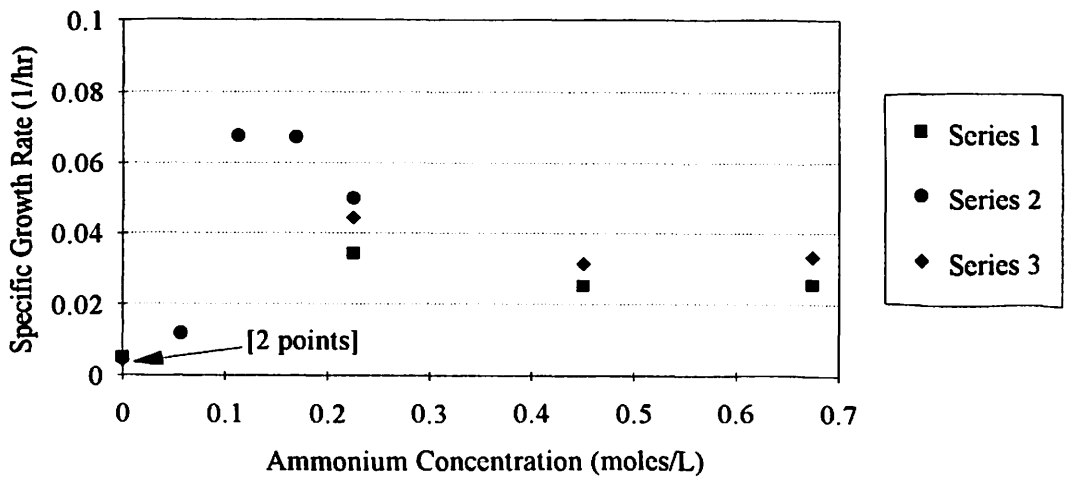

Figure 22. Specific Growth Rate Versus Ammonium Concentration

These results suggest growth inhibition begins where ammonium concentrations exceed 0.17 moles/liter. The optimal range for growth (0.1 to 0.2 M) is not the same range that is optimal for high constant respiration rates. In fact, ammonium concentrations favoring high constant BOD rates are relatively inhibitory to specific growth rate. Specific growth rate inhibition is a phenomenon that was also observed by Goel and Gaudy (1969). It also is evident from these results that optimization based on specific growth rates would create conditions favoring rapid growth but not necessarily the conditions which favor sustained growth to the point where highest constant rate respiration results. Constant rate over a long term is what is responsible for the bulk of substrate degradation. Thus, it appears that nutrient levels which contribute most to high, sustainable rates of constant respiration are most desirable for composting.

Temperature Test Results

The temperature study was set up as two sets of tests, one at the standard concentration of Evans' nutrient medium (low nutrient), the other at a nutrient concentration eight times higher (high nutrient) corresponding to the ammonium concentration with the highest observed μ in the previous nutrient experiment. Table 13 shows the rate results of this series.

TABLE 13

TEMPERATURE TEST RESULTS FOR
TWO CONCENTRATIONS OF AMMONIUM

Test #	Temp. (C)	Low Nutrients[a]			High Nutrients[b]		
		Constant BOD Rate		μ	Constant BOD Rate		μ
		Average[c]	Max. Dev.[c]	(1/hr)	Average[c]	Max. Dev.[c]	(1/hr)
361p7	25	0.028	0.002 (7%)	0.132			
361p53	25	0.041	0.013 (31%)	0.013			
361p25-26	35	0.178	0.016 (9%)	0.013	0.153	0.022 (14%)	0.220
361p28-29	40	0.060	0.012 (20%)	0.008	0.128	0.010 (8%)	0.126
361p31-32	45	0.025	0.016 (65%)	0.002	0.044	0.022 (51%)	0.016
361p34-35	50	0.033	0.003 (11%)	0.011	0.028	0.006 (20%)	0.047

[a] 0.014 M ammonium
[b] 0.112 M ammonium
[c] mg O_2/(gm VS x hr)

The effect that temperature had on microbial rates was significant. As may have been expected, the effect was most significant in the high nutrient set where specific growth rates were most dramatically affected. There were only small differences in constant BOD rates.

Controls which were sterilized ultimately developed a small measure of oxygen uptake, up to a maximum of 5 mg/gm VS in the 50 °C test, in contrast to active tests which ranged up to 67 mg/gm VS total BOD. The purpose of the sterile controls, which was realized, was to demonstrate that BOD in non-sterile reactors was not substantially a result of chemical oxidation.

An interesting point about the tests run at the temperatures of 45°and 50°C was that they began experiencing uptake about 100 hours before they terminated. This suggests that, since 50°C is near the beginning of the thermophilic range, a few thermophilic bacteria indigenous to the sludge may have gradually grown in population and finally exerted a measurable BOD. Because the tests terminated, it is not determinable

whether their respiration rates would have continued to improve with time. In a static compost pile, if temperatures were unregulated and allowed to rise into the thermophilic range, the mesophiles would gradually give way and be supplanted by the thermophiles. The results of this study are not sufficient to conclude that the mesophilic range is preferable over the thermophilic range for the biodegradation of hydrocarbons.

Constant Rate Analysis

Figure 23 shows the respiration rate profile for temperature for compost with a low level of nutrients (0.014 M NH_4^+). The first low nutrient constant respiration rate at 25°C was repeated because of the test's short duration (361p7 came out of the moisture content series) so a second test was run which resulted in a similar rate. Average rates are highest at 0.178 mg oxygen/(gm VS x hr) and drop precipitously above 40°C. 40°C is the upper end of the mesophilic range. The singular optimal temperature from these results for low nutrient levels is 35°C. Unfortunately, a test was not run at 30°C to indicate whether that temperature is as favorable for growth. Interestingly, the rates at 25°C were not high, despite being within the mesophilic range.

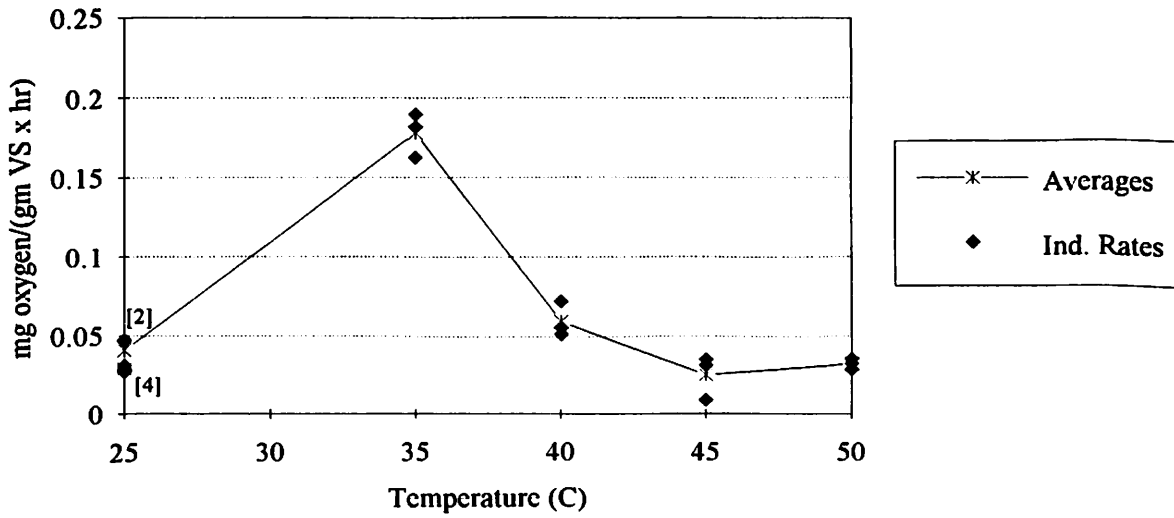

Figure 23. Constant Rate BOD vs. Temperature: Low Nutrients

A test from the nutrient series was the design model for the temperature series with high nutrient levels. That test's rates were to be used at the 25°C temperature. However, a mistake was made in this series and an incorrect moisture content was employed in the make-up of these tests (67% MC was mistakenly employed for 67% CC_{chips}). As a result, comparison of these results with the 25°C nutrient test was not deemed appropriate so it was omitted from Figure 24. For the high nutrient level (0.112 M NH_4^+), 35°C was the temperature most favorable to microbial activity. Whereas 40°C was limiting in the case of low nutrients, it appears to favor it the higher nutrient level. Perhaps the plenitude of nutrients allowed a rapid establishment of species adapted to 40° C, which may not have occurred under conditions of low nutrients. In both the low and high nutrient series, temperatures above 40°C appear inhibitory to microbial activity.

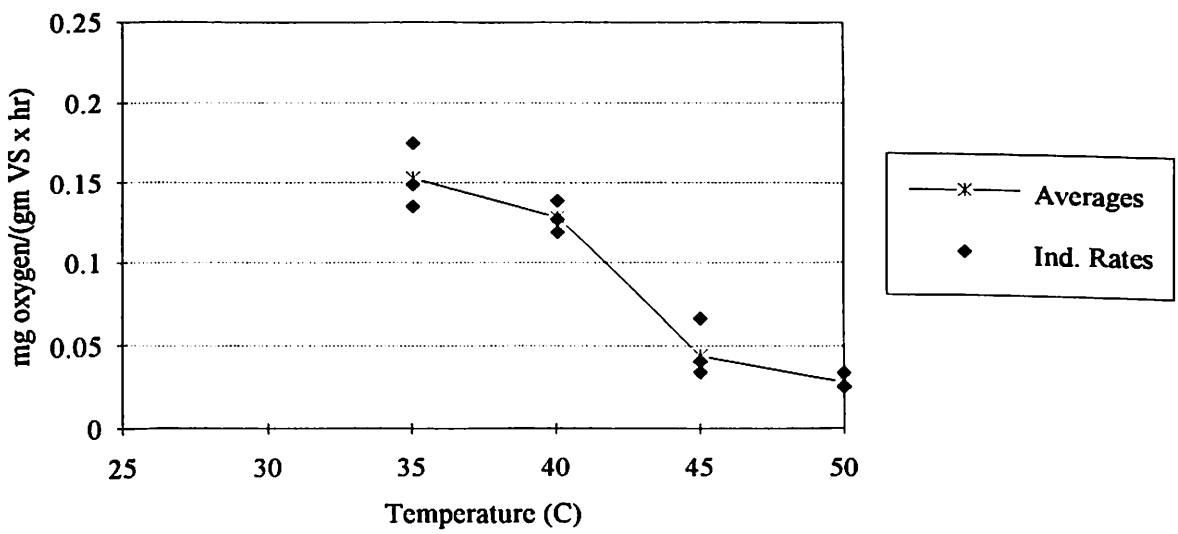

Figure 24. Constant Rate BOD vs. Temperature: High Nutrients

Specific Rate Analysis

Temperature had a dramatic effect on microbial growth rates in tests with high concentrations of nutrients. High nutrient levels also caused the data to be very amenable

to analysis for μ. The maximum value for specific growth rate was 0.22/hr in the high nutrient, 35°C test and was the highest observed μ among all series of tests. It was almost twice the value of any other growth rate in the series. Specific growth rates were sharply limited by temperature as they approached 45°C.

Two vastly disparate growth rates were obtained at 25°C in the low nutrient series. The test with the higher growth rate, 361p7, was from the moisture content series. The method of nutrient addition to the test may have been responsible for that high rate. 361p7 received 1.00 ml of Evans' concentrate which often contained suspended, undissolved salts. 361p53 received the standard dilute Evans' media. Both were calculated to receive the same stoichiometric amount of nutrient. Possibly too much nutrient was added to 361p7 as suspended salts. Its higher growth rate lasted less than 10 hours, and following the growth phase, its constant respiration rate was consistent with that observed in the repeat test. Figure 25 is a summary graph of the specific rate results.

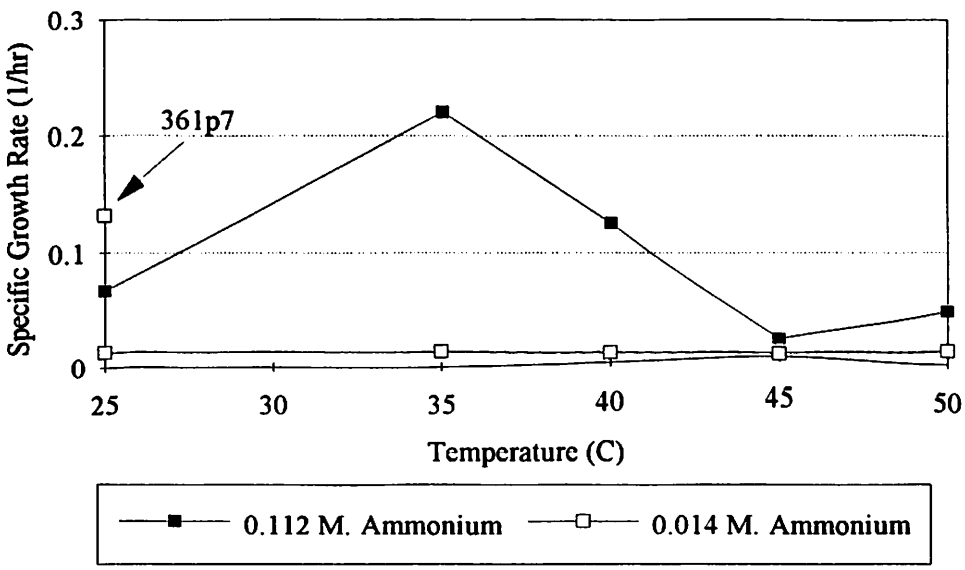

Figure 25. Specific Growth Rate vs. Temperature

Compaction Test Results

Compaction tests were run in triplicate for two different BA/S ratios, each at two degrees of compaction, 10% and 20%. The compaction tests for each BA/S ratio were compared to uncompacted tests of the same ratios from the BA/S series. Three sets of tests were limited to the results of two reactors each because leaks occurred during the course of the tests. Interpretation of the data was further complicated by two software interruptions, one of which occurred during the period of exponential growth. Never the less, following restoration of the data, rate determinations were obtained. Table 14 shows the those results.

TABLE 14

COMPACTION TEST RESULTS

BA/S Ratio (vol/vol)	Test #	Compaction (%)	Constant Rate BOD		μ (1/hr)
			Average[a]	Max. Dev.[a]	
1.1/1	361p42[b]	0%	0.061	0.011 (17%)	0.034
	361p50	10%	0.050	0.007 (15%)	0.027
	361p51	20%	0.047	0.011 (24%)	0.025
3.3/1	361p40[b]	0%	0.061	0.011 (18%)	0.011
	361p48	10%	0.048	0.004 (9%)	0.050
	361p49	20%	0.051	0.001 (2%)	0.050

[a] mg oxygen/(gm VS x hr)
[b] test from BA/S series

Constant Rate Analysis

These tests generally showed small differences in the magnitude of their average constant BOD rates, but were essentially the same within the ranges of variability in individual rates. Based on observations of previous series, there may have been a great

advantage in utilizing higher nutrient concentrations to stimulate activity to greater levels to better ensure that compaction (restricted permeability) would be the rate limiting factor.

At a BA/S ratio of 1.1/1, there appears to be a slight downward trend in average constant rate BOD with compaction, shown in Figure 26. However, considering the different source of sludge used for the previously performed uncompacted (0%) tests, their rates may not be repeatable using the same sludge employed in the compacted tests. A variance in rate repeatability, possibly due to use of different containers for sludge, has been observed previously in the results of both the hydrocarbon compaction and BA/S ratio experiments. At these low levels of nutrient concentration (<0.07 M ammonium), 20% compaction cannot be absolutely concluded to be more restrictive to microbial activity than 10% compaction.

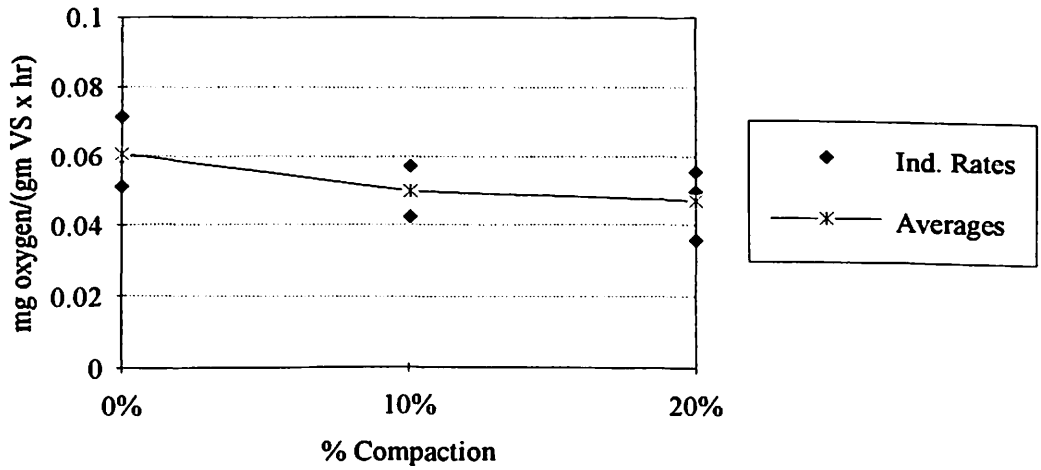

Figure 26. Constant Rate BOD vs. Compaction at BA/S = 1.1/1

At a BA/S ratio of 3.3/1, constant rate BOD averages were also nearly within the ranges of rate variability, as shown in Figure 27. The same concern holds true here as with the previous compaction set regarding variability in rates and possible differences in sludge source containers. Constant BOD rates are essentially the same between 10% and

20% compaction. It cannot be concluded on the basis of these results that a BA/S ratio of 3.3/1 is superior to 1.1/1 for resisting the effects of compaction.

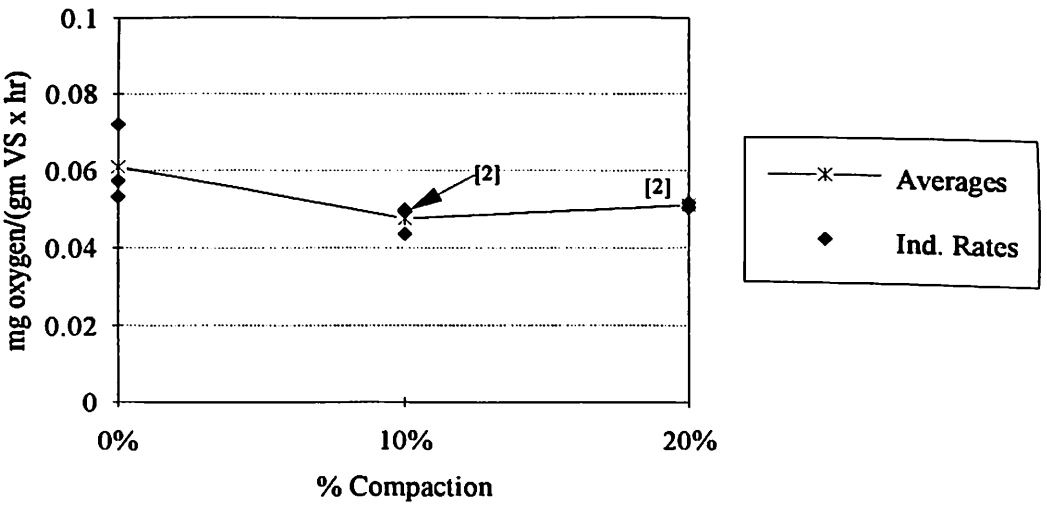

Figure 27. Constant Rate BOD vs. Compaction at BA/S = 3.3/1

Specific Rate Analysis

The specific growth rate results for the BA/S ratio tests are shown in Figure 28. The average specific growth rates for the 1.1/1 ratio tests display a very slight declining trend. The averages at the 3.3/1 ratio display no obvious trend. In view of these results it can only be concluded that increasing compaction has no discernible effect on growth rates under these conditions. One of those conditions was a low concentration of nutrients (~ 0.01 M NH_4^+), which may have limited the extent of exponential growth before compaction did.

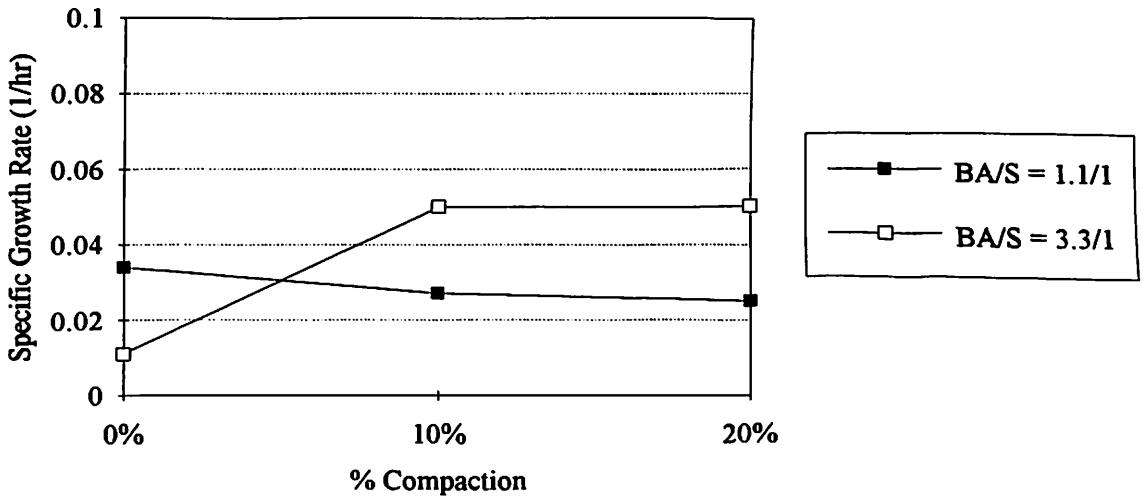

Figure 28. Specific Growth Rate vs. Compaction

Optimal Conditions Test Results

Oxygen uptake results from the optimal conditions test and its permutations were initially measured on the basisi of the total mass of volatile solids present in the sample (hydrocarbons and wood chips). The mass of sludge and hydrocarbons was held constant (except the compaction permutation). The proportion of volatile solids to hydrocarbons (VS/HC) was constant through four of the seven permutations. There were two additional tests based on specific growth rates which had a VS/HC ratio different from the optimal test. The effect of a greater mass of wood chips in one test compared to another was to dilute the actual BOD, resulting is lower BOD per unit mass VS.

Since the purpose of permutations was for comparison to a base condition (best combination) test, a means of normalizing BODs to a common mass was required. Since the mass of hydrocarbons was constant through all the tests but one (compaction), hydrocarbon mass seemed an appropriate basis on which to compare oxygen demand. That basis was predicated on the assumption that microbial activity on the bulking agent was minimal compared to that on hydrocarbons. This is demonstrated to be reasonable by

the low constant rate BOD for 0% HC in the earlier hydrocarbon concentration experiment (Figure 14). The graphs contained within this section are all in terms of oxygen uptake per mass hydrocarbon.

The results for the optimal conditions series of tests are shown in Table 15. One column shows the observed constant respiration rates, another shows the VS/HC normalization factor, and a third the resulting constant rate BOD per gram HC. The VS/HC normalization factor is simply the ratio of volatile solids mass to hydrocarbon mass in each respective test. Upon inspection, it becomes apparent that the "best conditions" test did not result in the highest constant rate of respiration. In fact, its rate was exceeded by no less than four tests on the basis of BOD/gm HC. The balance of the following discussion seeks to explain these apparent departures from expectations.

TABLE 15

RESULTS OF OPTIMAL CONDITIONS TESTS

Conditions of Test Showing Value of Permuted Parameter	Test #	Constant Rate BOD[a] (mg/(gm VS x hr))	Normalization Factor (VS/HC)	Constant Rate BOD[a] (mg/(gm HC x hr))	Specific Growth Rate[b] (1/hr)	Duration of μ (hr)
Best Combination	361p57	0.64	1.56	1.00	0.016	170
1.0 Molar Ammonia	361p59	0.11	1.56	0.16	0.020	60
83% Chip CC Test	361p60	0.58	1.56	0.90	0.015	220
25°C Temperature	361p63	*1.15*[c]	1.56	*1.80*	0.034	100
20% Compaction	361p58	0.20	1.56	0.31	0.012	180
15% HC (dwt)	361p62	*0.63*[c]	1.99	*1.26*	0.019	150
BA/S Ratio of 0.37/1	361p66	0.28	1.19	0.34	0.036	70
BA/S Ratio of 3.3/1	361p61	0.53	2.69	*1.41*	0.019	180
Specific Rate Opt. (35°C)	361p67	*0.89*[c]	1.99	*1.78*	0.035	90
Sp. Rate Perm. (25°C)	361p65	0.44	1.99	0.88	0.022	150

[a] Underlined figures exceed rate of best combination test.
[b] Comparisons only valid among series with same HC normalization factor.
[c] Tests selected for HC extraction.

Constant Rate Analysis

The constant respiration rate achieved by the "best combination" optimal conditions test was 0.64 mg oxygen/(gm VS x hr), 1.00 mg/(gm HC x hr). By use of the mineralization stoichiometry of 3.37 gm O_2/gm HC (Stegmann, *et al.* 1991), that constant rate, if maintained for 140 days, would result in the complete consumption of the available hydrocarbons. Some permutations, however, achieved higher rates. Figure 29 is the rate and cumulative uptake graph for the "best combination" test.

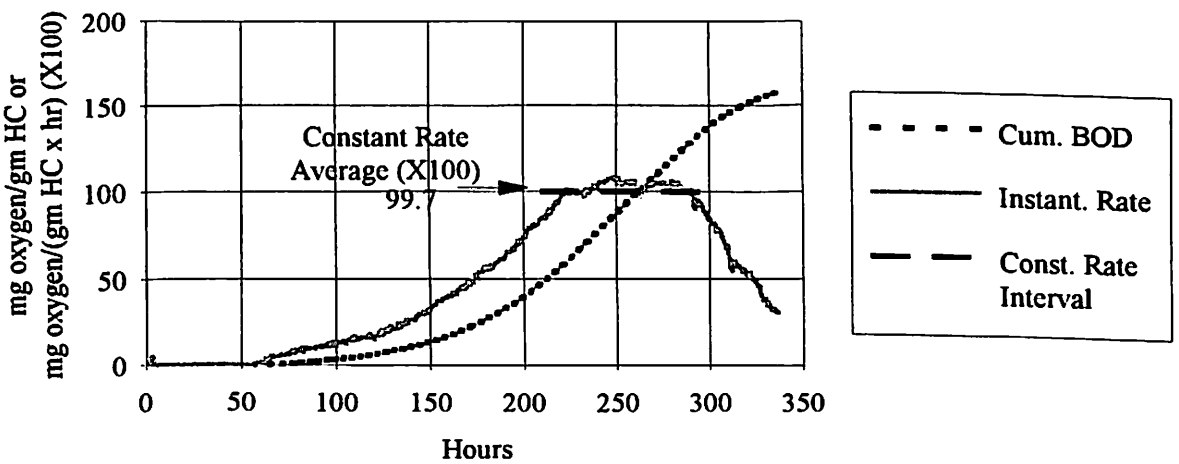

Figure 29. Best Combination Test: Rate and Cumulative Uptake

Nutrient. The 1.0 M ammonium permutation (Figure 30) attained only 16% of the rate of the "best combination" test, which was 0.5 M. The highest level of constant rate respiration in the earlier nutrient addition series was at an ammonium concentration of 0.674 M. Somewhere between 0.674 M and 1.0 M, ammonium concentration appears to become very inhibitory to microbial growth.

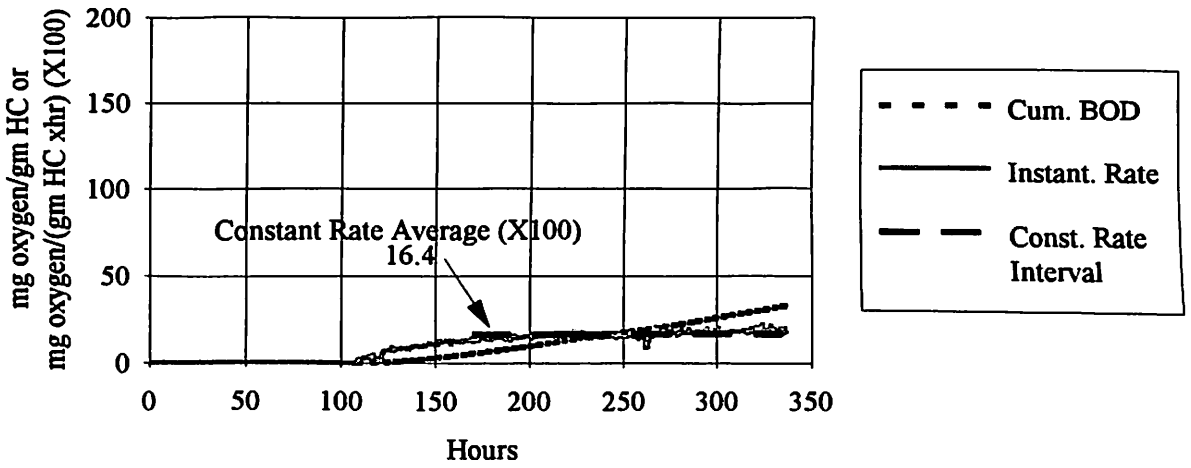

Figure 30. 1.0 M Ammonium Permutation: Rate and Cumulative Uptake

Moisture Content. The moisture content permutation (Figure 31) was 83% of chip container capacity (CC_c) versus 33% of CC_c in the best combination test. The constant BOD rate in the moisture permutation was 90% as high as in the optimum test. 83% CC_c was in the range of reduced constant BOD rates (47% of the highest rate) in the moisture content series (see Figure 18). This present result suggests that a viable moisture content range may extend from 30% to above 80% of CC_c.

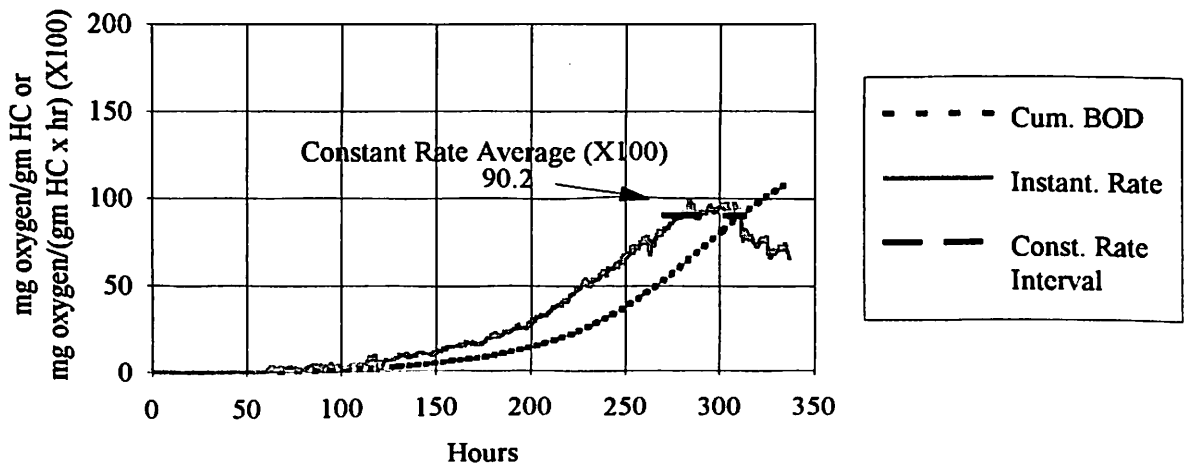

Figure 31. 83% Chip CC Permutation: Rate and Cumulative Uptake

Temperature. The high constant respiration rate at 25°C was a surprise (Figure 32). It was 80% higher than that of the "best combination" run at 40°C. The high nutrient (0.112 M NH_4^+) level temperature parameter test had been previously discounted from the results due to its water content being at variance with the series. There was no valid experimental result that suggested 25°C would favor high rates. At low nutrient concentration (0.014 M NH_4^+) the 25°C results were very low (earlier Figure 23). In view of those results, this test is not easily explainable. Its oxygen uptake rate peaked out at 2.42 mg/gm HC. Uptake never achieved a clearly defined constant rate, but fell off rapidly after achieving its peak. Some factor in the environment became totally expended (e.g. KOH) or waste product toxicity set in. This test suggests the strong possibility that at high (0.5 M) nutrient concentrations, the optimal temperature window extends down to 25°C. More comments concerning this test occur later in the discussion of the specific growth rate based 25°C permutation (361p65).

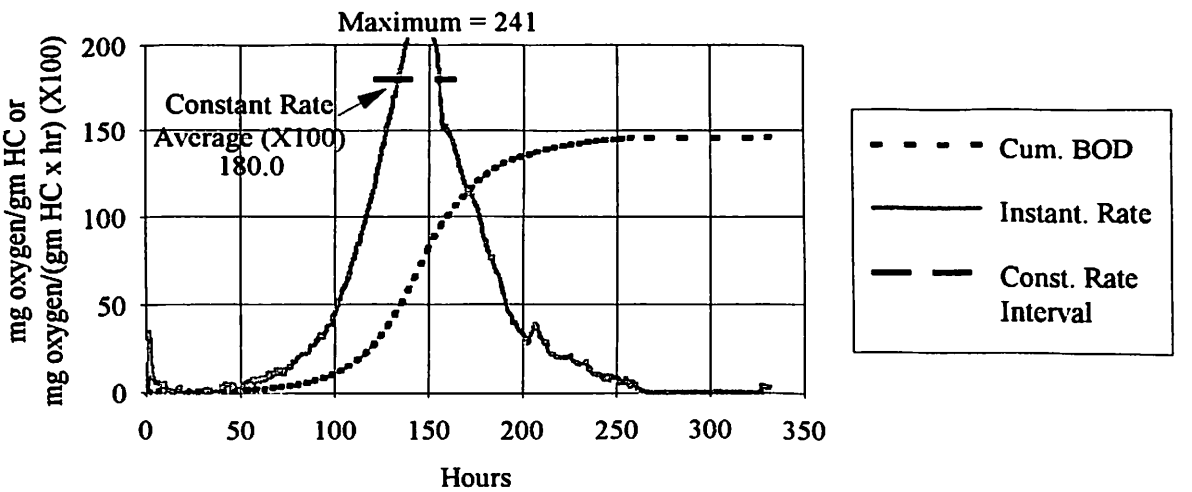

Figure 32. 25°C Permutation: Rate and Cumulative Uptake

Compaction. The 20% compaction permutation (Figure 33) attained a constant respiration rate only 31% of that of the "best combination" (0% compaction). Even so, the rate, 0.20 mg/(gm VS x hr), was 4 to 5 times greater than rates observed through the

compaction series of tests at identical BA/S ratio, due to the higher concentration of nutrients. The large reduction in rate from the optimal conditions test strongly supports a trend of decreased respiration rates with increased compaction at a BA/S ratio of 1.1/1.

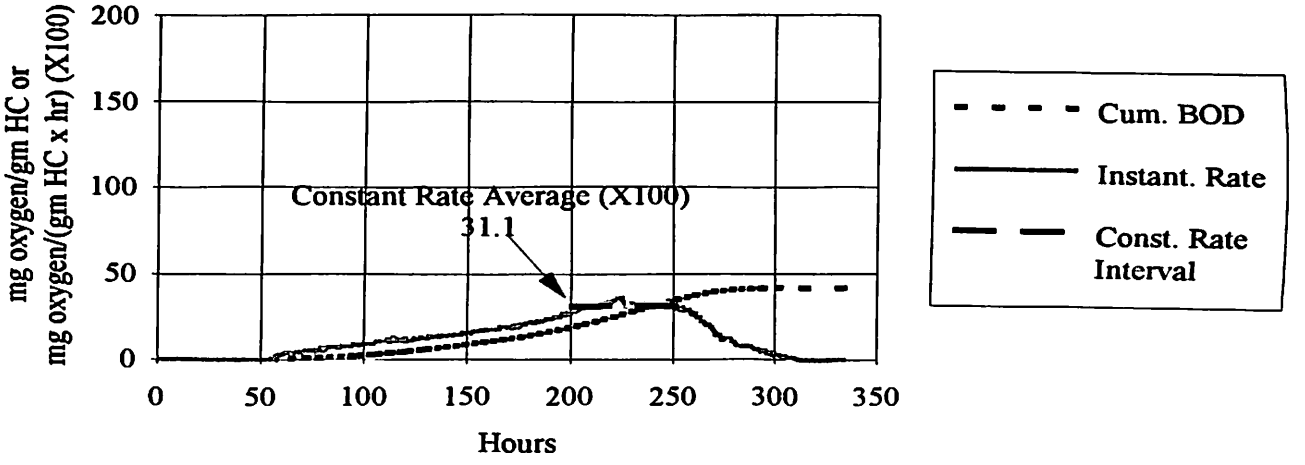

Figure 33. 20% Compaction Permutation: Rate and Cumulative Uptake

HC Concentration. Shown in Figure 34, the 15% hydrocarbon permutation showed a constant rate BOD of 1.26 mg/(gm HC x hr) and was 26% higher than the "best combination" test at 26% HC concentration. This result was expected for the reason that the "best combination" test utilized sludge at its original concentration, undiluted by anything but the bulking agent. The resulting 26% HC concentration was well above the optimal range (2% to 8%) identified in the HC concentration experiment. The addition of sludge inert material to the permutation served to dilute the hydrocarbon concentration in a direction favoring biodegradation. Beyond the addition of wood chips, dilution of sludge with soils can prove favorable for microbial activity.

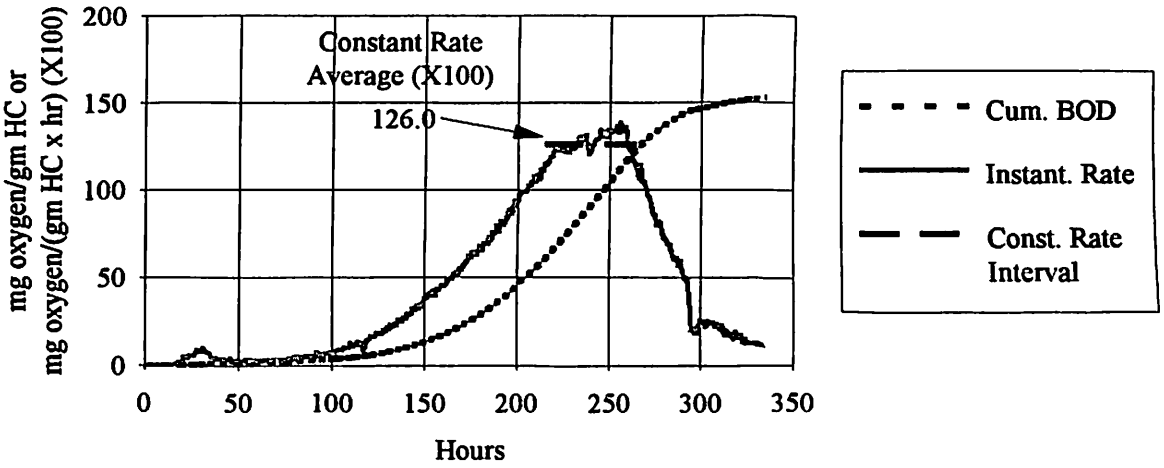

Figure 34. 15% HC Permutation: Rate and Cumulative Uptake

BA/S Ratio. Two permutations of bulking agent to sludge ratio were run. The permutation ratio of 0.37/1 (vol) was one third the ratio of the "best combination" test (1.1/1) and experienced a constant respiration rate only 34% of the "best combination" rate. It was clearly out of the optimal range of ratios and is shown in Figure 35.

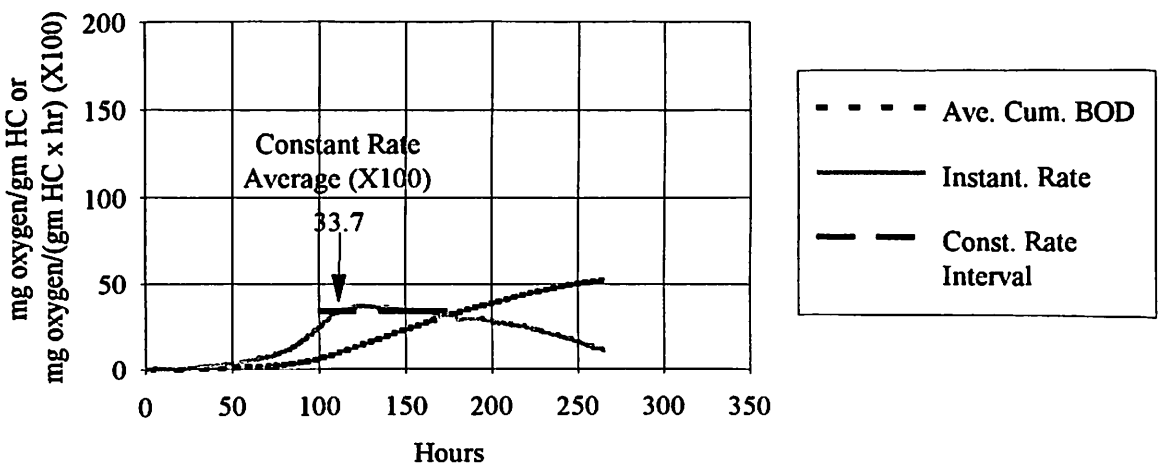

Figure 35. 0.37/1 BA/S Permutation: Rate and Cumulative Uptake

The other BA/S permutation was 3.3/1 (vol) and is shown in Figure 36, was three times the ratio of the "best combination". It resulted in a constant BOD rate 41% higher

than the "best combination" rate when normalized to hydrocarbon mass. One possible explanation for this has significance. The greater mass of chips in this test (3X) required three times as much make up liquid to maintain the 33% CC_c moisture requirement. The additional water required additional nutrients to maintain ammonia concentration at the desired constant 0.5 M. There was 54% more total moisture and nutrients available compared to the "best conditions" test. This additional liquid and nutrient constituted a "reservoir" for the microbes to draw from. Another contributing factor was the hydrocarbon dilution caused by the additional chips. HC concentration was 20% versus 26% in the "best combination". This result occurred because sludge mass was held constant in this series rather than chip mass, which was constant in the BA/S ratio series.

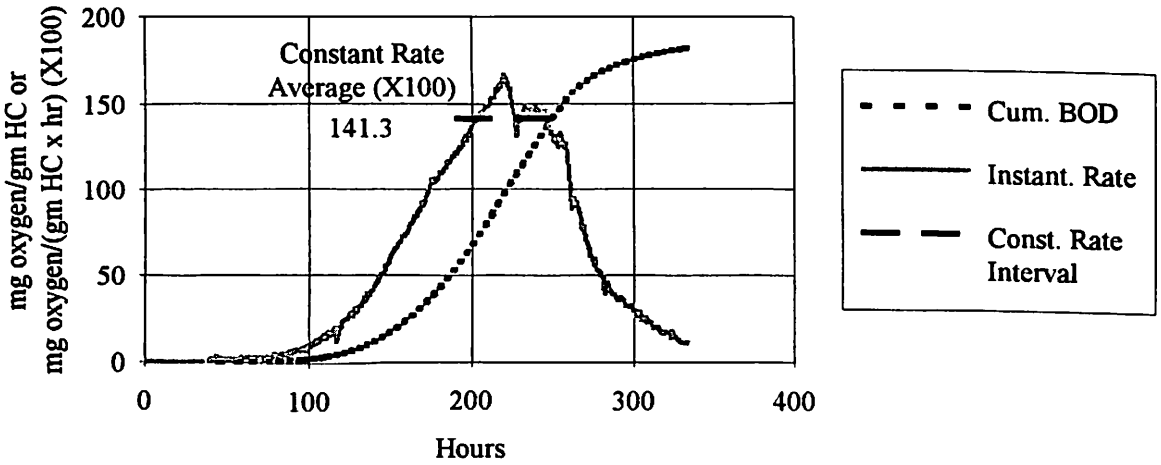

Figure 36. 3.3/1 BA/S Permutation: Rate and Cumulative Uptake

Specific Growth Rate Optimal Combination. An optimal growth conditions test was run based on the parameter values that had resulted in the highest specific growth rates, as outlined on page 47. The cumulative oxygen uptake plot for the optimal combination of specific growth rates is shown in Figure 37. The μ resulting from this test (0.038 hr^{-1}) was significantly less than some μ's in the parameter tests (e.g. 0.22 hr^{-1} for temperature, 0.18 hr^{-1} for water content) but was virtually equal to the best μ observed in

the *constant rate BOD* based optimal series. Interestingly, the constant rate BOD of this 35°C test was virtually equal to the highest from among those of the constant rate BOD based optimal series (see Table 15).

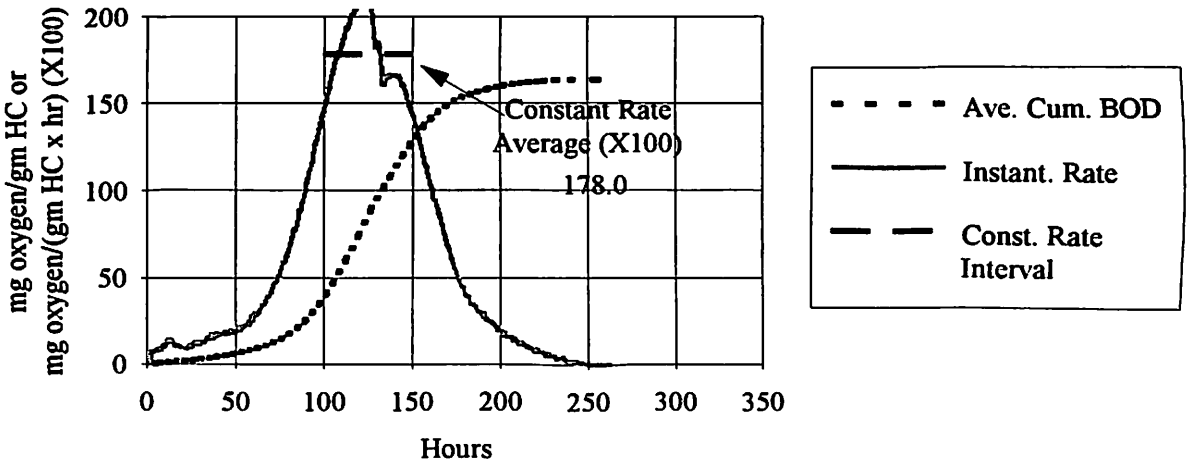

Figure 37. Specific Growth Rate Optimum: Rate and Cumulative Uptake

One μ permutation was run at 25°C (Figure 38). It resulted in a μ only 61% of that in the optimum test and is about what could have been expected based on the temperature results presented previously in Figure 25. The constant BOD rate was less than 50% of the constant rate in the optimal μ test. This was consistent with results observed in the *low* nutrient temperature parameter tests (earlier Figure 23). There was not a valid rate at 25°C in the high nutrient set to compare results to.

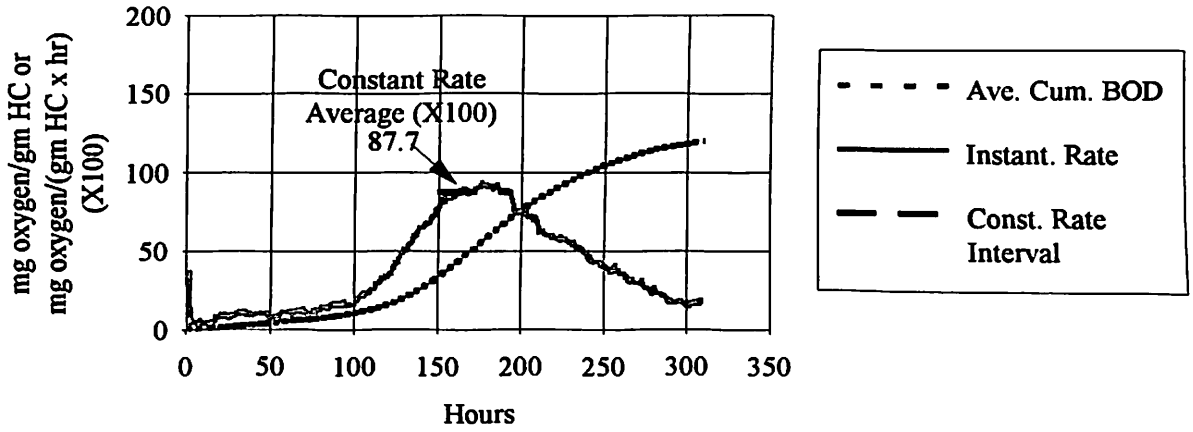

Figure 38. 25°C Sp. Rate Permutation: Rate and Cumulative Uptake

Optimization based on conditions favoring rapid growth were not successful in achieving a higher rate of growth, but was successful in achieving a high level of constant rate respiration. The constant BOD rate achieved was as high as any among the constant rate based optimal tests. This result was not entirely expected and begs the question of what caused this result. The answer may lie in the fact that the test was conducted at a temperature of 35°C. The overall results of these optimal tests and their permutations caused a re-evaluation of the temperature parameter data. This resulted in new maximum constant BOD rates at 35°C (as is currently reflected in Figures 23 and 24). Previously, 40°C had been believed to be optimum and was the value used for the optimal conditions constant BOD based test design.

Considering constant BOD rates among the constant BOD based optimal series tests, the "best combination" at 40°C was exceeded by the 25°C permutation. Among the specific growth rate based combinations, the 35°C test exceeded the 25°C permutation. Each pair of tests was identical in compostion, so it may be concluded that 35°C is the most favorable temperature of all, as shown by the results of the temperature experiment (Figures 23 and 24).

Extraction Results

Two tests and a control sample from the optimal conditions series were successfully extracted for hydrocarbons. The two tests were the "best conditions" and the specific growth rate based "optimal conditions" test. These selections were those which had achieved the highest cumulative BOD on a per mass volatile solids basis. Only later was it determined to compare rate results on a BOD/mass HC basis. Table 16 compares cumulative BODs for each test on the basis of both volatile solids and hydrocarbons. Different HC normalization factors are representative of different mass ratios of bulking agent to hydrocarbon.

TABLE 16

OPTIMAL TESTS CUMULATIVE BOD

Conditions of Test	Test #	Cumulative BOD (mg/gm VS)	Normalization Factor (g VS/g HC)	Cumulative BOD (mg/gm HC)
Best Combination	361p57	***101***	1.56	158
1.0 Molar Ammonia	361p59	21	1.56	33
83% Chip CC Test	361p60	72	1.56	112
25°C Temperature	361p63	***94***	1.56	147
20% Compaction	361p58	29	1.56	45
15% HC (dwt)	361p62	77	1.99	153
BA/S Ratio of 0.37/1	361p66	43	1.19	51
BA/S Ratio of 3.3/1	361p61	67	2.69	180
Specific Rate Opt. (35°C)	361p67	***105***	1.99	209
Specific Rate Perm. (25°C)	361p65	66	1.99	131

Tests with underlines selected for extraction.

The "best combination" tests lost 19.7% of their hydrocarbons over 14 days (end of test). The specific rate optimum tests lost 21.8% over 10 days (cessation of BOD exertion). The measured losses in mass of hydrocarbons from the extractions were

compared to oxygen delivered during respirometry. Stegmann, *et al.* (1991) used a formula for determining the oxygen requirement for the complete mineralization of a hydrocarbon to carbon dioxide and water (Equation 10), or theoretical oxygen demand. The formula assumes the hydrocarbons are all straight chain alkanes. Stegmann applied it to a diesel fuel and obtained the ratio 3.37 gm O_2/gm HC. The formula was applied in this work to the mole percentages of the 48 carbon numbers identified by GC type analysis of the sludge hydrocarbons. The result was a calculated oxygen requirement of 3.48 gm/gm HC. This ratio was used to calculate the theoretical oxygen demand of the removed compost hydrocarbons. These data are shown in Table 17. The data in the right most column reflect measured BOD as a percentage of the removed hydrocarbon's theoretical oxygen demand. They show there was a great deal more hydrocarbon missing from the samples than could be attributed to mineralization by measured BOD. For example, the average loss for the constant respiration rate "best combination" tests was 2.68 grams HC. According to the mineralization ratio, this amounts to 9.33 grams oxygen. In fact, the average oxygen delivered was only 2.09 grams, or 23% of the requirement. The discrepancy could easily be attributed to physical loss of HC during transfer and extraction or simply to the mineralization ratio being incorrect for this suite of hydrocarbons. Stegmann, *et al.* (1991) elaborated on similar discrepancies in the results of their work. They attributed the unaccounted loss of hydrocarbons to absorption by organic material within the compost and to incomplete solvent extraction. The range of BOD as a percentage of theoretical oxygen demand in their work was 37% to 48%. In this work it is 22% to 23%. Another reasonable explanation is that much of the hydrocarbon is not being mineralized. Rather than being oxidized by oxygen to form CO_2, a large proportion of carbon was incorporated into biomass. This was most significant during the exponential growth phase. This biomass carbon escaped extraction and contributed to the observed loss in hydrocarbons, but was not reflected as oxygen consumption. Cell synthesis during exponential growth certainly must contribute to the high hydrocarbon

removal rates observed for these tests. Because cell synthesis occurs predominantly through the period of short term exponential growth, these high hydrocarbon removal rates (0.17 to 0.27 gm HC/day) are not necessarily applicable for extended periods of constant rate BOD respiration.

TABLE 17

COMPARISON OF HC LOSS AND OXYGEN UPTAKE

Conditions	Test #	Average Net Loss (gm HC)[a]	Percent Reduction	O_2 Consumed per gram HC Removed	BOD as % of Theoretical. O_2 Demand[b]
Best Combination	361p57	-2.68	-19.7%	0.79	23%
Sp. Rate Optimum	361p67	-3.00	-21.8%	0.75	21%

[a] Does not take into account the possibility of head space O_2 utilization
[b] Mineralization ratio of 3.48 gm O_2/gm HC

Discussion of Results

Defining Moisture Content

Many researchers have discussed compost moisture content in terms of mass percentage. Stegmann, *et al.* (1991) employed the notion of "total water capacity", defined as the mass of water held per unit mass dry material. This is essentially equivalent to the notion of container capacity. The use of overall moisture content on a mass basis for compost must be questioned. The problem lies in the fact that an oily sludge compost is composed of at least three distinct substances having greatly varying densities. The masses of hydrocarbon, inert material, and bulking agent each enter into the expression for moisture content. Any number of mixtures of these substances can be made to total a constant mass by simply varying the proportions, i.e., one compost can consist of a low mass of BA, a high mass of soil, and be saturated with a specific amount of water.

Another compost of identical total dry mass may consist of a high mass of BA, a low mass of soil, have the same mass of water added, and yet be far from saturation. These two composts would still have the same mass percentage moisture content, yet totally different distributions of water within their matix. As is suggested by the constant BOD rate results, the degree of saturation can be responsible for large differences in levels of microbial activity. Thus, mass percentage moisture content does not represent the water content of a compost in a way which can be correleted well with its effect on microbial activity. It is suggested that container capacity be employed in compost research and design as the measure of water content. Container capacity depends on the relative proportions of the materials in the mixture and their own water holding abilities. Each compost, and each BA/S variation of a compost, has its own unique container capacity.

Growth Rate Versus Constant Rate Respiration

Water Content. One thing evident from the results presented in this chapter is that environmental conditions which favor high rates of microbial growth do not necessarily favor high rates of constant respiration. This is true for both the parameters of water content and nutrient concentration. A water content of 100% of CC_{chip}, while perhaps best for rapid microbial growth, is not in the best range (30%-83%) for sustaining high constant rate BOD. With this in mind, the initial stage of a compost process might benefit from a 100% $CC_{compost}$ water content to facilitate rapid growth of biomass. As water content drops with evaporation, it will descend into the range favoring higher constant respiration rates.

Nutrients. The case of nutrient concentrations is opposite that of water content. Ammonium concentrations favoring rapid growth rates are lower than those which favor the attainment of high constant levels of respiration. This implies that nutrient concentrations favoring rapid growth are not sufficient to sustain growth beyond a certain

point. At that point, additional nutrients could cause growth to continue at the same high rate.

Since the nitrogen which is required by bacteria is incorporated into biomass, it follows that it will be consumed most during the exponential growth phase. Once a steady state (constant rate respiring or rate limited) population is achieved, the requirement for supplemental nitrogen drops significantly. Whatever nitrogen is required to replenish and maintain the cell population is available through endogenous respiration or from other substrate (bulking agent). Nitrogen (and other nutrient) availability then is mainly critical during the growth phase and once a population has established itself to the limitations of its environment, less supplemental nutrients are needed. From this discussion it appears that for composting, nutrients are initially necessary only in a quantity sufficient to establish the population to the point where its growth becomes unavoidably limited by some other mass transfer rate (oxygen, HC surface area). On the other hand, the speed at which this growth occurs is a function of the resulting concentration of added nutrients, and excessive concentrations appear to greatly retard growth. Respirometry tests can determine the limit of microbial growth for a particular compost design, and nutrient supplements for bench or field scale tests can be tailored to that limit to avoid excessive, rate-inhibiting applications of ammonium. To further avoid inhibition of growth and to maximize constant rate BOD, nutrient supplements can be added in increments through the exponential growth period to maintain that window of concentrations most favorable for growth, or at least to keep the concentration below that (1.0 M) which becomes inhibitory to high respiration rates.

Beyond this, traditional nitrogen determinations made on the basis of a C:N ratio of 25:1 would result in inhibitory concentrations of ammonium when hydrocarbons become a significant percentage of the compost mass, such as would result from high TPH sludge (e.g., ammonium added to the "best combination" test on the basis of C:N = 25:1 would result in a concentration of nearly 2.0 M).

Overall, environmental conditions identified by constant rate BOD evaluations are recommended over those identified by use of specific growth rate. The exponential growth phase is of short term duration whereas constant rate, "steady-state" respiration ideally persists (with appropriate amendments) until substrate is depleted. This "steady-state" oxygen uptake rate is what is ultimately sought to be maximized and maintained because it is the long term "workhorse" rate which accomplishes the bulk of hydrocarbon degradation over time. It is proportional to the substrate utilization rate, in this case 3.48 gm O_2/gm HC utilized, which in turn can be used to predict the time required to degrade the hydrocarbon substrate.

Suggestions for Further Research

While this project resulted in some definitive determinations with respect to respirometry and composting, it opened up several issues for investigation which may have interesting possibilities.

Specific Growth Rate and Growth Duration Maximization

An issue of special interest to the author was the possibility of specific growth rate based optimization. It turned out that in series with nutrient supplements, there was an inverse relationship between specific growth rate and the duration of exponential growth. Simply put, the greater the growth rate, the shorter its duration, the lesser the growth rate, the longer its duration. The combination of the two, growth rate and the time of its duration, resulted in the constant rates of respiration which followed exponential growth. To illustrate this point the logarithmic population equation describing exponential growth is utilized:

$$ln(N/N_O) = \mu t \quad \text{so that when} \quad N_O = 1, \quad N = e\mu t \tag{16}$$

N_O is the original microbial population in the sludge. It is set to unity (1) and is presumed the same for tests made up with equal amounts of sludge from one container. Utilizing the observed durations (t) of exponential growth from tests and their corresponding graphically estimated specific growth rates (μ), the population sizes at the end of their exponential growth phases can be estimated. The resulting N is nothing more than a factor than indicates how many times the population has increased over its starting size, considered as unity. Figure 39 shows a graph of the resulting N's, herein termed biomass factors, versus their corresponding constant BOD rate results from the series of optimal conditions tests.

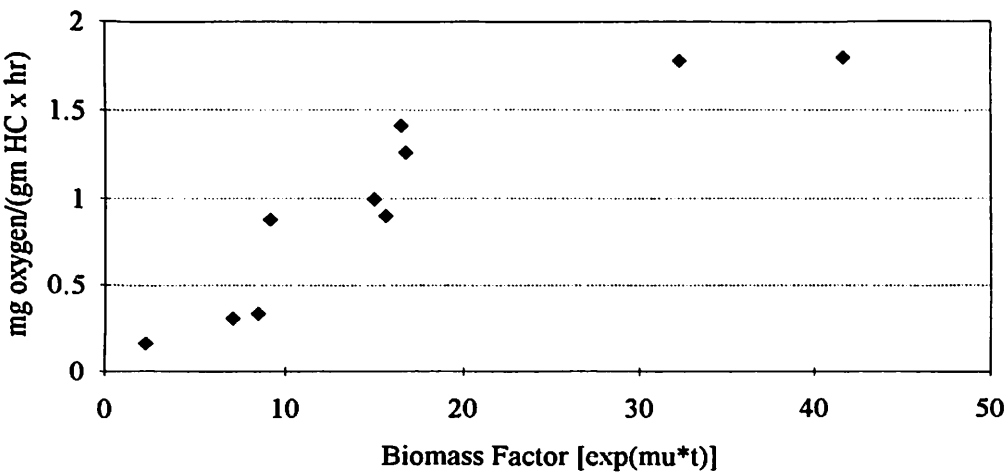

Figure 39. Constant Respiration Rate versus Calculated Biomass Size: Optimal Series of Tests

This graph makes it apparent that there is a strong correlation between size of microbial population (biomass concentration) and the resulting constant BOD rate that occurs at the end of exponential growth, as there should be according to Equation 10. Further, it can be concluded that any factor or physical change in a parameter which increases a growth rate *and/or* extends its duration would naturally result in a larger

biomass concentration and therefore a higher constant BOD rate. This is desirable for the concurrent increased rate of substrate utilization (hydrocarbon degradation).

It is believed that optimization based upon maximization of specific growth rates and extension of the duration of exponential growth can result in earlier, more rapid increases of biomass to concentrations higher than what might be ordinarily realized. This in turn could result in very high respiration and degradation rates. It is conceivable that a "high rate" composting process may be designed whose time to substrate depletion would be only a matter of weeks. This process could be tested using respirometry and a series of successive parameter evaluation tests. The parameter value for the test which results in the highest calculated biomass factor (from μ and it's duration, t) would be incorporated in the next parameter series, and so on through all parameters.

Long Term Respirometry

Long term studies could be done if much more KOH could be suspended in the reactors. This could be done with wide mouthed reactors and larger KOH cups. The purpose of extended duration tests would be to assess the longer term viability of compost cultures and see if highly optimized constant respiration rates can be maintained through to hydrocarbon depletion.

Incremental Nutrient Addition

It would be interesting to investigate whether incremental additions of nutrients, designed to maintain the narrow window of 0.10 to 0.23 M ammonium concentration, would result in sustaining the duration of highest exponential growth rates beyond that which results from a single dose. This idea could be tested using respirometry to monitor exponential growth. When growth rates stop increasing, additional moisture and nutrients could be added through a septum in the reactors. It would also be of interest to attempt to maintain the highest uptake rates therein achieved as constant levels of respiration.

Container Capacity Studies

Container capacity measurements could be made for all sorts of field materials and crop residues in order to determine those which might be best suited for composting. It would also be of interest to see how container capacity varies across the spectrum from 100% sludge to 100% bulking agent. Indications from this study are that the two individual container capacities are not simply additive.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Summary

The problem addressed by this study was whether respirometry was a suitable method for optimizing the environmental parameters affecting biodegradation of hydrocarbons in an oily sludge compost. A series of compost formulations were designed to test the effects of individually varying parameters on microbial activities as measured by oxygen uptake rates. The parameters investigated included hydrocarbon concentration, bulking agent to sludge ratio, moisture content, nutrient concentration, temperature, and compaction. Oxygen uptake was analyzed in two ways, on the basis of specific growth rates equated to uptake and on the basis of "steady state" or constant rate BOD respiration. Constant rate BOD generally was easier to determine and more significant an indication of process efficacy, and was employed as the basis for optimal range selection. The optimal parameter values indicated by the results of the parameter tests were combined in one test as a "proof case". The high concentration of oil in the sludge resulted in compost with a 18.7% hydrocarbon concentration on a wet weight basis. Permutations of the "proof case" were run and varied parameters individually away from their optimum values. Several additional permutations separately varied parameters towards more favorable conditions (lower HC%). Analysis of oxygen uptake from the "proof case" and permutations showed the "proof case" exhibited the highest level of constant rate BOD compared to negative permutations, with the exception of the temperature permutation. Subsequent re-analysis of the original temperature data yielded

an optimal temperature different than was employed in the "proof case", suggesting the permutation was actually in a favorable direction. The positive permutations resulted in the highest constant BOD rates, indicating the highest degree of microbial activity. The results of the "proof case" and it's permutations validate the use of respirometry as a evaluatory tool for optimization of the environmental parameters affecting composting.

General Conclusions Concerning Respirometry

Overall, the results demonstrate that respirometry, under the proper conditions, is a sensitive and reliable method for measurement of compost oxygen uptake rates and is well suited for parameter optimization. This is concluded from the following general points.

1. Compost samples, relying solely on microbial populations indigenous to oily sludge, when adequately amended with nutrients, were sufficiently active to produce consistent patterns of oxygen uptake among replicate tests.
2. Addition of nutrients significantly increased the degree of microbial activity, though excessively high concentrations were inhibitory.
3. The generation of repeatable oxygen uptake data was adversely affected by use of sludge from different containers and shelf ages. Sludge should be kept in sealed, refrigerated containers to preserve its original numbers and distribution of microbial species.
4. Constant rate BOD analysis is a viable method for evaluating the effects of a varying environmental parameter and can be used as the basis for optimization.
5. Respirometry is well suited for optimizing the process of composting an oily sludge.

General Conclusions for Composting an Oily Sludge

1. High petroleum content sludge is capable of being biodegraded by means of composting without requiring significant dilution of hydrocarbons (compost TPH was 18.7%).
2. The optimum water content for specific compost is best measured as a percentage of its saturated condition, or container capacity. Each particular bulking agent to sludge ratio has a unique container capacity.
3. Nutrients are essential for high rates, and amendments are best made on the basis of target nitrogen or ammonium concentrations for the total water available in the compost. The most favorable range is 0.4 M to 0.7 M ammonium.
4. An important consideration for selection of a bulking agent, in addition to its ability to resist compaction, is its capacity for holding water.
5. Substantial and rapid degradation of hydrocarbons occurs at temperatures within the mesophilic range, specifically 25°C to 40°C.

Specific Conclusions for Farmington Sludge

1. On a wet weight basis, the Farmington sludge is comprised of approximately 24% hydrocarbons, 20% moisture, and 56% inert material.
2. 100 to 1000 bacteria per gram were indigenous to the sludge.
3. Gravimetrically determined losses of hydrocarbon from compost in two "proof case" optimized tests were 19.7% over 14 days and 21.8% over 10 days.
4. The parameter ranges determined by respirometry to be most favorable for the composting of Farmington sludge are described as follows:

a. For wood chips as bulking agent, the favored bulking agent to sludge ratio is 1:1 by volume or higher. The actual ratio employed can be determined by the requirement to resist the effects of compaction (restricted permeability) due to pile height or the need for additional water content, and may be 3:1.

b. Hydrocarbon concentration can be subjugated to that which results from addition of bulking agent. High hydrocarbon concentrations are not prohibitive to microbial activity, though lower concentrations in the range of 2% to 8% favor it. Sludge with high hydrocarbon concentrations can be diluted with soils of low concentrations with a beneficial impact on activity.

c. The optimal moisture content range is from 30% to 83% of wood chip container capacity, though higher levels of water content may be desirable to help control pile temperature and provide larger amounts of nutrients without exceeding a specific concentration. Water content of up to 100% of wood chip container capacity is not prohibitive to microbial activity. Through evaporation, moisture content will gradually diminish to more favorable levels.

d. A complete nutrient amendment may be added on the basis of ammonium concentration. 0.4 M to 0.7 M ammonium is the most favorable range of concentrations. The mass of nutrient amendment to be added should be determined on the basis of concentration in the total water available in the compost (compost container capacity). Ammonium concentrations approaching 1.0 M are inhibitory to growth and high levels of respiration. To increase the available nutrients in compost without exceeding a limiting ammonium concentration, additional water may be required. This can be done by employing a higher percentage of $CC_{compost}$ for water content and/or the use of a higher BA/S ratio (additional bulking agent) maintained at an equivalent or higher percentage of container capacity.

e. Among the temperatures investigated in this study, the mesophilic range of 25°C to 40°C is most favorable for hydrocarbon degradation. This is not an indication that higher thermophilic temperature are unfavorable. Temperatures above 50°C were not tested in this experiment.

f. There was no significant effect on microbial activity resulting from up to 20% compaction on compost with a either a 1.1/1 or 3.3:1 BA/S (vol) ratio at low concentrations of nutrients (<0.01 M ammonium). At high concentrations of nutrients (0.5 M ammonium), a significant reduction in uptake rates (~70%) was experienced with 20% compaction.

A summary of the most favorable ranges for the composting of Farmington sludge as determined by both constant rate BOD and specific growth rate analysis is presented in Table 18.

TABLE 18

OPTIMAL CONDITIONS FOR COMPOSTING FARMINGTON OILY SLUDGE

Compost Parameter	Constant Rate BOD Basis Best Range	Specific Growth Rate Basis Best Range
BA/S Ratio	1/1 and higher	Inconclusive
HC Concentration	2.0% to 8.0%	Inconclusive
Water Content	30% to 83%[a]	100%[a]
Nutrient Conc.[b]	0.4 to 0.7 M	0.1 to 0.23 M
Temperature[c]	25°C to 40°C	35°C to 40°C
Compaction[d]	less than 20%	Inconclusive

[a] as wood chip container capacity for solvent extracted chips
[b] as NH_4^+ concentration (moles/liter)
[c] based on 25°C permutation in optimal series
[d] for BA/S of 3.3/1 and <0.01 M NH_4^+

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APPENDIX A

FORMULATIONS AND PROCEDURES

CONTAINER CAPACITY

Container Capacity (CC) is defined as the ratio of the mass of water retained against gravity to the dry mass of the material tested. The following steps detailing the determination of container capacity are adapted from Cassel & Nielson.

1) The subject material is loaded and shaken down loosely into a 15 cm. tall container with a perforated bottom and known mass.

2) The container is placed in a deep pan of shallow water and allowed to wet through capillary action. Over the next 12 hours the pan is gradually filled to submerge the container to saturation.

3) Once saturated, the container is set atop another to allow free drainage of water to air. The top is loosely covered to prevent evaporation.

4) Following six hours of drainage, the container and material are weighed.

5) The container is then placed in a drying oven at 105°C for 24 hours.

6) The container and dried mass are weighed to determine the mass of water which was previously held by the material. The dried material mass is determined by subtraction of the original container weight.

7) Container capacity is the ratio of the mass of water held to the mass of dried material and has units of g H_2O/g dwt material.

EVANS' MINERAL SALTS MEDIA

(Rosenberg and Gutnick, 1981)

Compound	g/liter
$(NH_4)_2SO_4$	1.00
K_2HPO_4	1.00
$MgSO_4 \cdot 7H_2O$	0.30
$CaCl_2$	0.10
$FeSO_4 \cdot 7H_2O$	0.02

Adjust to pH 7.0

DREWS' TRACE ELEMENT SOLUTION*
(10X concentration)

Compound	mg/liter
$MnCl_2 \cdot 4H_20$	100
$CoCl_2$	20
$CuSO_4$	10
$Na_2MoO_4 \cdot 2H_2O$	10
$ZnCl_2$	20
LiCl	5
$SnCl_2 \cdot 2H_2O$	5
H_3BO_3	10
KBr	20
$BaCl_2$	5
EDTA	8,000

Add 1 ml per liter of mineral salts media.

*The above formulation is for 1 liter of solution. It is a tenfold concentration, as set forth in Reichenbach and Dworkin (1981), of the original Drew's formulation. The tenfold concentration was used throughout this study.

MICROBIAL ACTIVITY ON WOOD CHIPS VERSUS HYDROCARBONS

The design of this compost respirometry experiment was predicated on minimizing microbial activity on the bulking agent. Preliminary respirometry tests employing a sludge derived inoculum and natural wood chips in stirred Evans' media showed oxygen uptake activity of 0.11 mg/gm chips/hr. The activity of nutrient amended sludge flattened across the bottom of respirometer bottles was 0.10 mg/gm/hr. In view of these very similar rates, solvent extraction of the wood chip resins and oils was employed to reduce their potential for microbial activity.

Wood chips were extracted for 24 hours in a 1.75 liter Soxhlet vessel with a 50/50 mixture of methanol and chloroform. Following extraction, the chips were spread in a pan

beneath a lab hood and air dried for 24 hours. They were next packed into 250 ml containers and sealed with foil. 24 hours prior to use, these containers were sterilized in an autoclave at 120°C for 30 minutes.

When tested in the same way as the natural chips for microbial activity, the 50/50 solvent extracted chips showed a constant oxygen uptake rate of 0.022 mg oxygen/(gm x hr); about an 80% reduction in activity, shown in Figure 40.

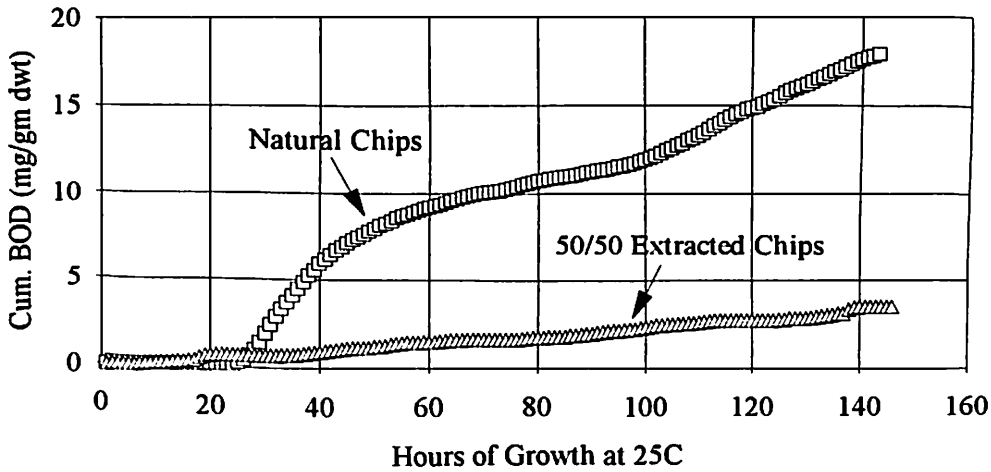

Figure 40. Average Microbial Activity on Natural and 50/50 Extracted Wood Chips

In order to more effectively remove the degradable substances in the chips a sequential extraction was performed employing first methanol and then chloroform, each for 24 hours. The resulting chips were termed "double extracted". Activity on these chips is shown in Figure 41 and was measured at 0.037 mg oxygen/(gm x hr), actually higher than before. No reduction of microbial rates were achieved by sequential extraction of the chips. However, there was approximately 50 hours of lag time before the inception of measurable oxygen uptake on the double extracted chips. The increased total time of extraction did reduce substances which were initially available in the 50/50 extracted chips. As a result of this effort, all chips employed in subsequent respirometry tests were double extracted.

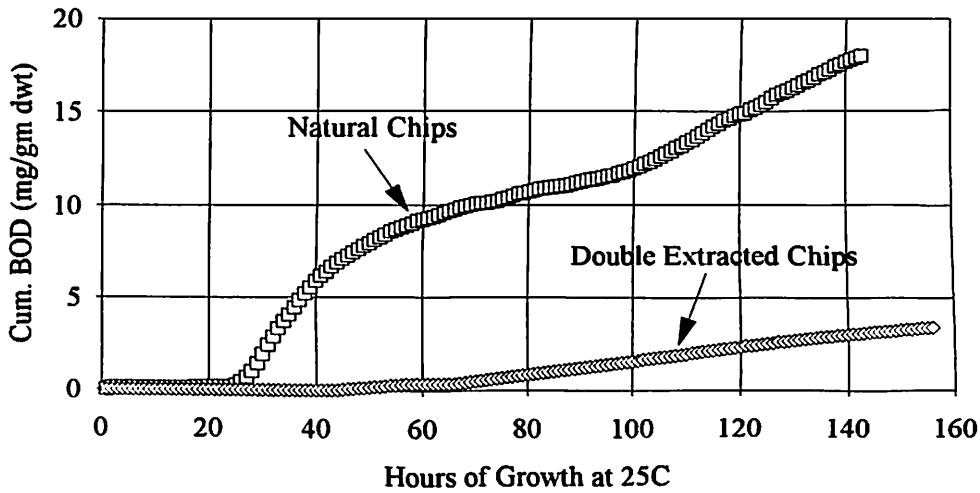

Figure 41. Average Microbial Activity on Natural and Double Extracted Wood Chips

Another set of tests were done to allow a better comparison of activities on wood chips and sludge hydrocarbons. The previously described chip tests were performed in 150 ml of stirred Evans' media. The chips gave the mixture the consistency of a coarse slurry. Uptake rates were controlled, but not necessarily limited, by mass transfer of oxygen into the aqueous phase. Bacteria were widely distributed in the mixture and should have had ample room for growth on the large surface area of the wood chips. In comparison, the earlier test on sludge exposed only a single flat surface to the reactor head space and was not continuously mixed. To provide a more equal footing for comparison of activities, a test was devised which used centrifuged sludge hydrocarbons in stirred Evans' media. In this test the hydrocarbons, which had the appearance and viscosity of a grease, mostly adhered to the stir bar with some free floating globs. Despite this, the average oxygen uptake rate was 4.8 mg/(gm HC x hr), indicating that in a liquid, microbial activity on pure hydrocarbon is significantly (>160X) higher on a mass basis compared to wood chips.

This comparison indicated that in a liquid media the degradability of the sludge hydrocarbons was significantly greater, 130 times more than that of the extracted chips (Figure 42). This was in spite of limited surface area compared to the chips. More rapid dissolution of hydrocarbons into the aqueous phase in the stirred reactors, and thus greater bioavailability, could have influenced the uptake rate. It appeared that most of the solid hydrocarbons were covered by a scum of bacteria. What effect this had on the rate of dissolution is unknown.

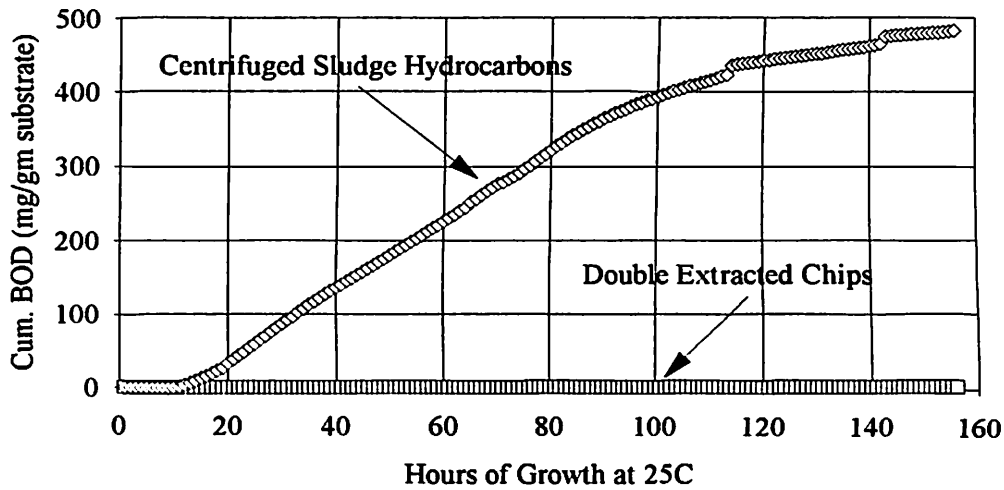

Figure 42. Microbial Activity on Sludge Hydrocarbons and Extracted Wood Chips in Stirred Evans' Media

Another test run later in conjunction with a series evaluating the effect of hydrocarbon concentration on microbial rates provides another useful point. The 0% HC concentration test was run with 16.00 gms of chips and 5.60 gms. of sludge derived inert material at a moisture content of 62%. This had 2.0 ml of sludge derived "bacterial seed" supernatant added to it and was essentially a wetted chip mixture in an air environment. The resulting activity was at a constant oxygen uptake rate of 0.015 mg/(gm x hr). This value falls below the two previous values for microbial activity on extracted chips in a *liquid* environment, 0.022 and 0.037 mg/(gm x hr). Mass transfer of oxygen into the aqueous phase must not be a limiting factor at these low levels of uptake.

An implication of the forgoing comparison of activies in liquid versus air is that a microbial population, subsisting on pure hydrocarbons on a matrix surrounded by air (compost), wetted with a thin film of moisture containing enough nutrients and room for growth, would support the same high levels of activity as seen in submerged, stirred conditions. Though this argument is by no means conclusive, it does give an indication that the activity of bacteria on hydrocarbons in test sample compost may be significantly higher than on the extracted wood chips used for bulking agent.

What is not known is the nature of the interplay between hydrocarbons and wood chips when they are in intimate contact with one another. The presence of hydrocarbons may further minimize the activity on wood chips by covering their surface. On the other hand, cometabolism of wood constituents may take place, or rapid growth on hydrocarbons may engender further colonization on wood chips.

For these reasons, oxygen uptake throughout this experiment was evaluated on the basis of total volatile solids (VS) in the compost sample. Volatile solids included the mass of hydrocarbons, always calculated as 24% of the mass of sludge employed, and the dry mass

of wood chips, which were 99.7% volatile. The masses of wood chips employed throughout the parameter test series' were the same (i.e. 16.00 gm. dwt) and for each series, came from one batch extraction. Only in the optimal conditions series of tests were wood chip masses varied from the regimen described above, and those were made to meet the design conditions of the series, while the mass of sludge hydrocarbons remained constant.

POTASSIUM HYDROXIDE CO_2/O_2 DEMAND

Wetted potassium hydroxide pellets are used as a CO_2 absorbent in the respirometer bottles. The CO_2 absorption capacity of the KOH is important because it determines the amount of oxygen which can be delivered to the reactors. The absorption reaction is:

$$CO_2 + 2KOH \rightarrow K_2CO_3 + H_2O$$

The measured mass of 50 KOH pellets was 4.81 grams. The purity of the KOH was 87.8% and its molecular weight was 56.1 gm/mole. A standard reactor load through early series of tests consisted of ten pellets equaling 0.845 grams or 0.0151 moles KOH. According to the stochiometry, 0.00755 moles of carbon dioxide could be absorbed by that amount of KOH. This indicated, since oxygen and carbon dioxide have identical molar volumes, that 0.00755 moles, or 0.242 grams of oxygen would be delivered to the reactor over the same period to replace the CO_2. Thus, the oxygen delivery ratio for 87.8% purity KOH was 0.242 gm O_2/10 pellets or 0.251 gm O_2/gm KOH.

The important consideration is that KOH should be present in sufficient quantity so as not to preclude or inhibit the clear development of a constant respiration rate plateau on cumulative oxygen uptake curves. This is simply to ensure that the growth and/or rate limiting influence of the parameter variable under investigation will manifest. During the cell synthesis reaction, not all molecular oxygen is oxidized to CO_2, but ends up as produced H_2O. Thus, oxygen can be delivered to a reactor beyond the amount which can be attributed to the KOH reaction with CO_2. In the nutrient series of tests, delivered oxygen exceeded KOH allowable oxygen by a factor of from 3.0 to 3.6. Since the highest observed total BOD through this experiment was 3,000 mg, KOH quantity should be sufficient to allow for up to, say, 1/3 that amount, or 1.00 gm O_2. The required amount of KOH can be calculated as follows:

$$\#\ \text{KOH tablets} = 1.00\ \text{gm}\ O_2\ \text{X}\ \frac{1\ \text{KOH pellet}}{0.0242\ \text{gm}\ O_2} = 41.3$$

The practical capacity limit for double suspended KOH cups is not much above 30 pellets total due to production and absorption of water, particularly at elevated temperatures. This limit places a constraint on the amount of compost material that can be utilized for a test In general, most tests will get by suitably with 30 pellets of KOH. Any time that

oxygen uptake rates peak, drop precipitously and approach zero, oxygen starvation due to KOH depletion must be considered as a possible cause.

The respirometer maintains a constant amount of oxygen in the headspace of the reactor (20.9% by volume). When the KOH is exhausted, respiration may well continue until head space oxygen, calculated to be 0.139 grams O_2 for a 500 ml reactor at STP, is near or at depletion. During this stage, instead of being absorbed, evolving CO_2 merely replaces the oxygen in the headspace as the O_2 is taken up. This amount of oxygen usage is unrecorded by the respirometer's computer. It can have an impact on cumulative BOD based evaluations because it may provide up to 19% more oxygen than the KOH allowed amount for 30 pellets. In other words, biodegradation of hydrocarbons can continue beyond the cessation of oxygen deliveries to the reactor.

CALIBRATION OF THE RESPIROMETER

In order for the amount of oxygen delivered to a reactor to be accurately measured, the delivery valve has to be calibrated to a known volume of oxygen This is accomplished by the draining of a measured volume of water from a 100 ml buret connected via tubing to a respirometer port. N-Con Systems, Inc. proprietary respirometer software program, C-Tox, has a step by step procedure which facilitates valve calibration and saves the resulting valve coefficient for use in the mass determination of delivered oxygen. Each oxygen delivery valve of the respirometer is individually calibrated to obtain a unique valve coefficient.

Several items of data are necessary to be known for accurate determination of valve coefficients. These include: 1) elevation, 2) barometric pressure, 3) ambient room temperature, 4) regulator or line pressure. These values are entered into the computer when requested by the software.

Ideally, a portable barometer located in the same room is best used for determination of barometric pressure. On occasion, such as during stormy weather, it may be necessary to re-enter barometric pressure due to change over the course of the calibration procedure.

Oxygen line pressure is regulated to 10 psi. If calibration is done too rapidly, such as by running a steady stream of water from the buret, line pressure can drop by more than 1 psi, thus reducing the amount of oxygen which fills the valve. When delivered to the buret, that mass of oxygen will occupy less than 90% of the volume a 10 psi delivery would have, so too rapid of delivery results in too large a number of deliveries and too small a calculated mass of oxygen per delivery. Line pressure drop is best monitored by use of a mercury manometer attached to the delivery line. Be sure to provide an overflow apparatus to capture mercury in case of regulator failure.

The calibration procedure is to fill a calibration buret with water to the zero line. The buret top valve is closed and a respirometer port connection is made. The software

program is instructed to accuate the appropriate valve circuit whereupon the bottom valve of the buret is opened to allow drainage of water as discrete droplets. As water drains from the buret it exerts a partial vacuum on that port's piezometer. When sensed by the computer it responds with an oxygen delivery to the buret. Deliveries continue until 200 are made, whereupon the computer software issues a prompt to close the buret. The resulting volume reduction of water in the buret is noted (usu. 30-45 ml) and entered into the computer. The software, by means of the ideal gas law, calculates 1) an average mass for the oxygen contained by a valve's single delivery and, 2) a unique valve coefficient which is utilized for all subsequent oxygen mass determinations for tests on that port. Each port is re-calibrated until three sequential mass measurements agree to within 1% (typically two to three μg, oxygen mass usually ranges from 220 to 250 μg per delivery, depending on the valve).

OXYGEN DELIVERABILITY TESTS WITH SODIUM SULFITE

Anhydrous sodium sulfite is readily soluble in water and the sulfite ion quickly is soon oxidized to sulfate in the presence of oxygen. This compound offers an excellent means for checking the calibration of the respirometer's oxygen delivery system.

Approximately one gram of Na_2SO_3 should be added to 100 ml of de-ionized water in a reactor with a stir bar. One gram will suffice for about 25 hours of oxygen uptake at a bath temperature of 25°C. Longer periods of uptake may be desirable as in the example below. The rate of oxidation is controlled by the rate of mass transfer of oxygen across the air/water interface in the reactor and is greatly affected by temperature.

Figure 43 shows oxygen uptake histories for identical masses of 4.73 grams sodium sulfite, one at 25°C and one at 35°C. Na_2SO_3 had a molecular weight of 126.0 and was virtually 100.0% pure. The stochiometric requirement for oxygen was calculated to be 127 mg/gm sodium sulfite. The 25°C test consumed oxygen to within 5% of the stochiometric requirement and the test at 35°C was within 2%. The rate of delivery at 35° C was 3.25 times higher than at 25°C. A higher temperature has increased the rate of oxygen mass trasfer from air to water. This may have had an influence on the greater microbial rates observed in this experiment at a temperature of 35°C.

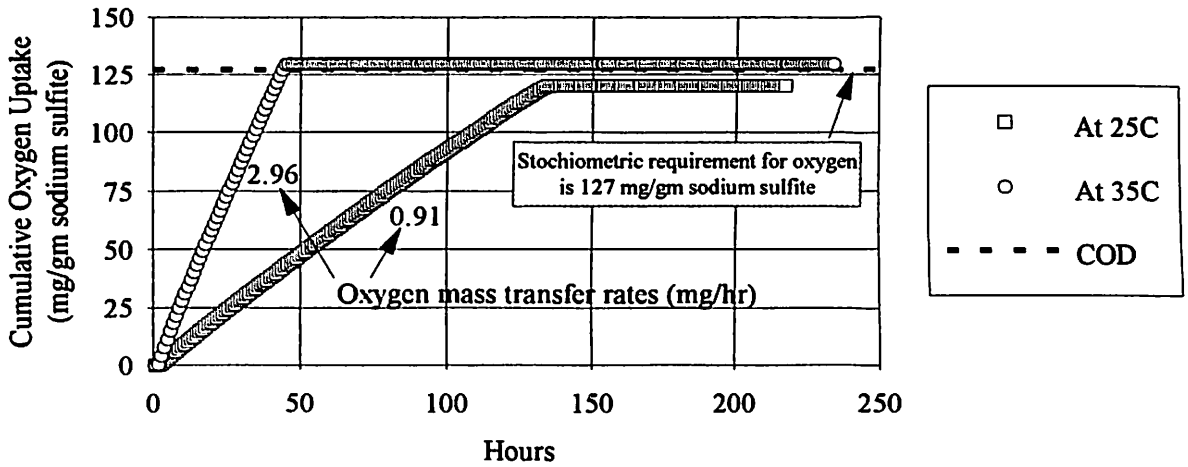

Figure 43. Oxygen Delivery Tests

An 30 hour calibration check can be run for all reactors simultaneously by using approximately one gram of sodium sulfite per reactor. The Comput-Ox program requires a minimum input value of 20, so use of 100 times the actual mass is convenient, but it must not be forgotten to multiply the results by 100. Ideally, all uptake plots should plateau at the same level.

APPENDIX B

TEST MAKE-UP TABLES

MAKE-UP OF HYDROCARBON CONCENTRATION TESTS

(in grams)

	% HC	TEST #	Mass B.A. (dwt)	M.C. Chips	Mass Sludge	Sludge Inerts	Added Inerts	Total Inerts	Sludge HC	Added HC	Total HC	% HC (Inert Basis)	Added Evans	Added Water (for inerts)	Total Water (a)	% M.C. (w/o HC)	% of CC Chips (b)	Nutrient Conc. (NH4 M.)
Series 1	0.0%	337p72	16.00	8%	0.00	0.00	5.60	5.60	0.00	0.00	0.00	0%	32.36	2.0 (c)	35.85	62%	71%	0.014
	2.5%	337p82	16.00	8%	2.31	1.29	4.30	5.59	0.55	0.00	0.55	9%	32.36	1.55	35.86	62%	71%	0.014
	5.0%	337p73	16.00	8%	4.74	2.66	2.95	5.60	1.14	0.00	1.14	17%	32.36	1.05	35.85	62%	71%	0.014
	7.5%	337p83	16.00	8%	7.30	4.09	1.51	5.60	1.75	0.00	1.75	24%	32.36	0.55	35.85	62%	71%	0.014
	10% (d)	337p70	16.00	8%	10.00	5.60	0.00	5.60	2.40	0.00	2.40	30%	32.36	0	35.85	62%	71%	0.014
	15.0%	337p74	16.00	8%	10.00	5.60	0.00	5.60	2.40	1.41	3.81	40%	32.36	0	35.85	62%	71%	0.014
	20.0%	337p71	16.00	8%	10.00	5.60	0.00	5.60	2.40	3.00	5.40	49%	32.36	0	35.85	62%	71%	0.014
Series 2	0.0%	337p104	16.00	7%	0.00	0.00	5.63	5.63	0.00	0.00	0.00	0%	31.96	2.0 (c)	35.16	62%	70%	0.014
	0.5%	337p94	16.00	15%	0.45	0.25	5.34	5.59	0.11	0.00	0.11	2%	32.16	1.91	37.05	63%	74%	0.013
	1.0%	337p93	16.00	15%	0.91	0.51	5.08	5.59	0.22	0.00	0.22	4%	32.16	1.81	37.04	63%	74%	0.013
	2.5%	337p92	16.00	15%	2.31	1.29	4.30	5.59	0.55	0.00	0.55	9%	32.16	1.54	37.05	63%	74%	0.013
	5.0%	337p91	16.00	15%	4.74	2.65	2.94	5.59	1.14	0.00	1.14	17%	32.16	1.05	37.05	63%	74%	0.013

(a) Total water includes moisture content of chips, sludge water, added water and Evans' solution.
(b) does not include sludge moisture
(c) seed inoculum
(d) 10% HC is base bondition

MAKE-UP OF BULKING AGENT TO SLUDGE RATIO TESTS

(in grams)

	Test #	% HC (dwt basis)	Mass Chips (dwt)	Sludge (wwt)	Mass HC	Sludge Inerts	Evans' Media (a)	Total Moisture	Moisture Content	% CC Chips (b)	Nutrient Conc. (M,)	B.A./S. Ratio (dwt to inert basis)	B.A./S. Ratio (dwt basis) (incl. HC)	B.A./S. Ratio (vol./vol.)
Series 1	337p97	5.0	16.00	4.00	0.96	2.24	23.67	25.67	57%	53%	0.014	7.14	5.0	32.9
	337p98	10.0	16.00	10.00	2.40	5.60	23.67	26.87	53%	53%	0.013	2.86	2.0	13.2
	337p99	15.0	16.00	20.00	4.80	11.20	23.67	28.87	47%	53%	0.012	1.43	1.0	6.6
	337p100	20.0	16.00	40.00	9.60	22.40	23.67	32.87	41%	53%	0.011	0.71	0.5	3.3
	337p102	30.0	0	176.23 (c)	42.30	98.69	0.95 (d)	36.20	20%	(no chips)	0.010	0/1	0/1	0/1
Series 2	361p22	1.0	16.00	0.69	0.17	0.39	23.67	25.01	60%	53%	0.014	41.00	29	191.0
	361p21	2.5	16.00	1.82	0.44	1.02	23.67	25.23	59%	53%	0.014	15.70	11	72.4
	361p20	5.0	16.00	4.00	0.96	2.24	23.67	25.67	57%	53%	0.014	7.14	5.0	32.9
	361p19	7.5	16.00	6.67	1.60	3.74	23.67	26.20	55%	53%	0.014	4.28	3.0	19.8
Series 3	361p40	20.0	16.00	40.00	9.60	22.40	23.67	32.87	41%	53%	0.011	0.71	0.50	3.3
	361p41	22.6	16.00	60.79	14.59	34.04	23.67	37.03	36%	53%	0.010	0.47	0.33	2.2
	361p42	25.7	16.00	119.04	28.57	66.66	23.67	48.68	30%	53%	0.007	0.24	0.17	1.1
	361p44	26.6	12.00 (e)	119.04	28.57	66.66	23.67	48.38	31%	69%	0.007	0.18	0.13	0.83
	361p45	27.7	8.00 (e)	119.04	28.57	66.66	23.67	48.08	32%	102%	0.007	0.12	0.08	0.55

(a) equal to 50% of chip CC

(b) Does not include sludge moisture

(c) to approximate the volume of other tests

(d) Sludge only case used 25X concentrated Evan's Media

(e) had decreased mass of chips rather than increased mass sludge due to volume constraints

MAKE-UP OF MOISTURE CONTENT TESTS

(in grams)

TEST #	Sludge Mass	% HC (dwt)	Chips (wwt) (a)	Evans' Conc. (b)	Added Water	Total Water (c)	% CC Chips	% CC Compost (d)	% Overall Moisture Content	Ammonia Conc. (M.)
361p2	10.00	10%	17.52	1.00	0	2.52	5.3%	12%	16	0.168
361p3	10.00	10%	17.52	1.00	13.28	15.8	33%	46%	43	0.043
361p4	10.00	10%	17.52	1.00	21.20	23.72	50%	67%	52	0.029
361p5	10.00	10%	17.52	1.00	29.12	31.64	67%	88%	58	0.023
361p6	10.00	10%	17.52	1.00	36.99	39.51	83%	108%	63	0.018
361p7	10.00	10%	17.52	1.00	44.91	47.43	100%	129%	67	0.015

(a) Chip dry weight equals 16.00 gms.
(b) Evans' 50X concentrate is 0.758 Molar w.r.t. ammonia
(c) Total water includes chip moisture, nutrient, and added water.
(d) 100% compost CC = 1.58 X dry mass chips, HC, and inert solids. For B.A./S. of 13.2/1 only.

MAKE-UP OF NUTRIENT ADDITION TESTS

(in grams)

	Test #	Nutrient Load: % Stoch. Req. (a)	Mass Sludge	Mass HC	% HC (dwt)	Chips (wwt) (b)	Mass of Nutrient Conc. (c)	Added Water	Total Water (d)	% CC Chips (e)	Overall Moisture Content	Resulting NH4 Concentr. (mole/L)
Series 1:	337p87	0%	10.00	2.40	10%	17.35	0.00	30.30	33.65	67%	58%	0.00
	337p88	33%	10.00	2.40	10%	17.35	10.03	20.90	33.71	67%	58%	0.225
	337p89	67%	10.00	2.40	10%	17.35	20.06	11.50	33.77	67%	58%	0.450
	337p90	100%	10.00	2.40	10%	17.35	30.09	2.10	33.84	67%	59%	0.674
Series 2:	337p105	8.3%	10.00	2.40	10%	17.69	2.51	27.61	33.67	67%	58%	0.056
	337p106	17%	10.00	2.40	10%	17.69	5.02	25.26	33.69	67%	58%	0.113
	337p107	25%	10.00	2.40	10%	17.69	7.52	22.91	33.69	67%	58%	0.169
	337p108	33%	10.00	2.40	10%	17.69	10.03	20.56	33.71	67%	58%	0.225
Series 3:	361p08	0%	10.00	2.40	10%	17.40	0.00	30.24	33.64	67%	58%	0.000
(repeat of 1)	361p09	33%	10.00	2.40	10%	17.40	10.03	20.84	33.70	67%	58%	0.225
	361p10	67%	10.00	2.40	10%	17.40	20.06	11.44	33.76	67%	58%	0.450
	361p11	100%	10.00	2.40	10%	17.40	30.09	2.04	33.83	67%	58%	0.674

(a) Based upon 100% conversion of HC to biomass

(a) Dried weight wood chips is 16.00 gm.

(c) Evans' 50X concentrate is 0.758 M. and has a density of 1.06 gm./ml.

(d) Total Moisture = Sludge water (2.00 gm), chip water (1.40 - 1.69 gm), nutrient concentrate/1.06, and added water.

(e) Does not include sludge water

MAKE-UP OF TEMPERATURE TESTS

(in grams)

Type of Test	Test #	Temp. (C)	Sludge Mass	% HC (dwt)	Chips (wwt)	Evans' Soln. (standard)	Evans' 50X Conc.	Added Water	Total Water	Overall M.C.	M.C. as % CC Chips	Nutrient Conc. (as NH4)
High Nutrient	337p107 (a)	25	10.00	10%	17.69	0	7.52	22.91	33.63	58%	70%	0.158
Low Nutrient	361p7 (b)	25	10.00	10%	17.52	0	1.00	44.91	49.37	67%	103%	0.014
Low Nutrient	361p53	25	10.00	10%	17.58	44.49	0	0	48.07	67%	100%	0.014
Low Nutrient	361p25	35	10.00	10%	17.58	44.49	0	0	48.07	67%	100%	0.014
High Nutrient	361p26	35	10.00	10%	17.58	0	7.52	37.40	48.07	66%	100%	0.112
Sterile	361p27	35	10.00	10%	17.58	0	7.52	37.40	48.07	66%	100%	0.112
Low Nutrient	361p28	40	10.00	10%	17.58	44.49	0	0	48.07	67%	100%	0.014
High Nutrient	361p29	40	10.00	10%	17.58	0	7.52	37.40	48.07	66%	100%	0.112
Sterile	361p30	40	10.00	10%	17.58	0	7.52	37.40	48.07	66%	100%	0.112
Low Nutrient	361p31	45	10.00	10%	17.20	44.87	0	0	48.07	67%	100%	0.014
High Nutrient	361p32	45	10.00	10%	17.20	0	7.52	37.78	48.07	66%	100%	0.112
Sterile	361p33	45	10.00	10%	17.20	0	7.52	37.78	48.07	66%	100%	0.112
Low Nutrient	361p34	50	10.00	10%	17.20	44.87	0	0	48.07	67%	100%	0.014
High Nutrient	361p35	50	10.00	10%	17.20	0	7.52	37.78	48.07	66%	100%	0.112
Sterile	361p36	50	10.00	10%	17.20	0	7.52	37.78	48.07	66%	100%	0.112

(a) from nutrient series
(b) from moisture series
(c) CC chips = 3.0 X dryweight chips

MAKE-UP OF COMPACTION TESTS

(in grams)

Test #	B.A./S. Ratio (vol./vol.)	B.A./S. Ratio (dwt/inert)	Compac-tion	Total Sludge	% HC (dwt)	Chips (wwt)	Evans' Soln. (stand.)	Added Water	Total Water	% CC Chips (b)	Overall M.C.	Resulting Nutrient Conc. (M.)
361p42 (a)	1.1/1	0.24/1	0%	119.04	26%	17.28	23.67	0	49	158%	31%	0.007
361p50	1.1/1	0.24/1	10%	184.50	26%	26.78	23.67	13.00	76	158%	31%	0.005
361p51	1.1/1	0.24/1	20%	199.99	26%	29.03	23.67	16.00	82	157%	31%	0.004
361p40 (a)	3.3./1	0.71/1	0%	40.00	20%	17.28	23.67	0	33	158%	41%	0.011
361p48	3.3./1	0.71/1	10%	88.00	20%	38.02	23.67	28.00	72	156%	41%	0.005
361p49	3.3./1	0.71/1	20%	96.00	20%	41.47	23.67	32.60	79	156%	41%	0.005

(a) tests from B.A./S. series run in normal reactors, others run in special oversized reactors

(b) Use of constant % chip CC results in equivalent moisture distributions between chips and sludge

MAKE-UP OF OPTIMAL CONDITIONS TESTS

(in grams)

Conditions of Test or Permutation	Test #	B.A./S. (vol./vol.)	Sludge Mass	Chips (dwt) (a)	Sludge Inert Solids	Added Inert Solids	HC Mass	% HC (dwt)	HC Conc. w.r.t. Inert Solids	Nutr. Soln. to add (b)	Make-up Water	Total Make-up Liquid	Total Compost Water	% CC Chips (c)	Overall M.C. (%)	Resulting Nutrient Conc. (M.)
Best Combination:	361p57	1.1	59.02	8.00	33.05	0.00	14.16	26%	30%	4.21	4.29	7.89	20.58	33%	27%	0.49
1.0 Molar Ammonia:	361p59	1.1	59.02	8.00	33.05	0.00	14.16	26%	30%	8.42	0.69	7.89	20.58	33%	27%	0.99
83% Chip CC Test:	361p60	1.1	59.02	8.00	33.05	0.00	14.16	26%	30%	6.67	14.03	19.73	32.42	83%	37%	0.50
25C Temperature:	361p63	1.1	59.02	8.00	33.05	0.00	14.16	26%	30%	4.21	4.29	7.89	20.58	33%	27%	0.49
20% Compaction: (d)	361p58	1.1	194.8	26.40	109.07	0.00	46.74	26%	30%	13.89	14.15	26.02	67.91	33%	27%	0.49
15% HC (dwt): (e)	361p62	1.1	59.02	14.00	33.05	36.11	14.16	15%	17%	8.20	19.68	26.69	40.05	33%	29%	0.49
B.A./S. Ratio of 0.37/1: (f)	361p66	0.37	59.02	2.67	33.05	0.00	14.16	28%	30%	1.08	5.64	6.56	18.66	83%	27%	0.14
B.A./S. Ratio of 3.3/1:	361p61	3.3	59.02	24.00	33.05	0.00	14.16	20%	30%	7.73	17.05	23.66	38.13	33%	35%	0.49
Specific Rate Opt. (35C)	361p67	2.0	59.02	14.00	33.05	0.00	14.16	23%	30%	2.74	32.05	34.40	47.75	83%	44%	0.14
Specific Rate Perm. (25C)	361p65	2.0	59.02	14.00	33.05	0.00	14.16	23%	30%	2.74	32.05	34.40	47.75	83%	44%	0.14

(a) Wood chip moisture content is 10%

(b) 79X Evans' concentrate is 2.41 Molar w.r.t. ammonia and has a density of 1.17 gm/ml.

(c) does not include sludge water

(d) Oversized reactor requires 250 ml + 20% volume.

(e) Added inerts required additional bulking agent to maintain 1.1/1 ratio. Also % CC Chip ignores additional moisture added for inert solids amendment.

(f) employed incorrect CC chips (83% instead of 33%). Difference is 27% over intended total moisture; 3.95 gm. over 14.61 gm.

VITA

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