AN INVESTIGATION OF TEMPERATURE EFFECTS ON DENITRIFYING BACTERIAL POPULATIONS IN A BIOLOGICAL NUTRIENT REMOVAL (BNR) SYSTEM

by

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(Abstract)

The goal of this research was to characterize the effects of temperature changes on the denitrification process in a biological nutrient removal (BNR) system. Specifically, there were three objectives. First, the effects of temperature changes on denitrification rates by a bacterial population from a BNR system were investigated. Next, the role which PHAs (poly-beta-hydroxyalkanoates) played in the denitrification process were examined. Finally, the effect of temperature changes on the production and consumption rates of PHAs was determined.

Sacrificial batch experiments were performed to assess the kinetic and chemical trends present in the denitrification process. Mixed liquor from the last anaerobic zone of a pilot scale BNR system was injected into vials. These vials were pre-purged with nitrogen gas in order to prevent dimolecular oxygen (O_2) from being entrained in the

mixed liquor. Next, the vials were placed on a shaker table for 30 minutes in order to allow all external COD to be consumed. Following this, each vial was injected with nitrates and various macronutrients. This process was repeated for three different sets of batch tests; each set was identical except for the added substrate. One set received no added substrate while the other two received either acetate or glucose. Vials were sacrificed over a period of three hours and analyzed for nitrate, phosphate, PHB (poly-beta-hydroxybutyrate), PHV (poly-beta-hydroxyvalerate), glucose and acetate.

These tests showed that specific denitrification rates in the batches with no added substrate were equivalent to or lower than rates in the other batch tests. This result was expected, since no readily biodegradable substrate (RBCOD) was available for the bacteria to utilize. Additionally, specific denitrification rates measured from unamended batch tests did not change significantly with changes in temperature. Furthermore, there were no predictable trends in PHA concentrations in any batch test, regardless of the substrate added. For this reason, it is believed PHAs were not being used as substrate for denitrification. In the case of the unamended samples, it is believed that the bacterial population were using slowly biodegradable COD (SBCOD) obtained from the pilot plant influent as substrate for denitrification.

Denitrification rates measured from acetate-amended batch tests exhibited no temperature dependency, similar to results from the unamended tests. It is believed that the acetate was being utilized by phosphorus removing (poly-P) bacteria during denitrification, since poly-P bacteria have been shown to perform well at lower temperatures, possibly due to a population shift within the poly-P community to more psychrophilic bacteria. Furthermore, it is believed that acetate was being utilized by a different pathway than is used for the production/consumption of PHAs.

During this study, glucose amended nitrate removal rates exhibited a significant temperature dependency, with specific denitrification rates decreasing with decreasing temperature. It is believed that glucose was utilized primarily by non-poly-P denitrifiers, since typical heterotrophic denitrifiers have been shown to exhibit similar temperature dependencies. Additionally, it is believed that glucose was preferentially consumed by these denitrifiers rather than poly-P organisms, since most poly-P bacteria would have to break the glucose down into volatile fatty acids (VFAs) before utilization.

Another finding of this research was that the ratio of PHB to PHV as internally stored substrates remained constant throughout each batch test, regardless of time and substrate added. Although the amount of each PHA varied between samples, their ratio remained essentially constant. Additionally, as the temperature changed, this ratio did not change significantly.

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CHAPTER ONE: INTRODUCTION

In recent years, research has shown that excessive nutrient loadings can lead to eutrophication of water bodies. In order to limit the contribution from point source loadings, many methods of nutrient removal have been investigated. In order to remove phosphorus and nitrogen from wastewater, biological nutrient removal techniques have been developed.

Alternating anoxic and aerobic conditions are used to eliminate nitrogen from wastewater. In this process, nitrate and ammonia are converted to nitrogen gas. In order to remove phosphorus biologically, alternating anaerobic and aerobic conditions are used. In this scheme, phosphorus is stored in the bacterial cell as polyphosphate granules and removed in the process of biomass wasting. Under anaerobic conditions, poly-P bacteria store substrate internally as poly-beta-hydroxyalkanoates (PHAs). These PHAs are then consumed under aerobic conditions to provide sufficient energy for polyphosphate granule formation, which leads to the removal of phosphorus from the wastewater.

In the past two decades, a great deal of research has been conducted to investigate the effects of temperature changes on biological nutrient removal systems. This work has contributed significantly to characterizing the effect of temperature on nitrogen removal systems. The impact of temperature on the performance of phosphorus removal systems, however, is not as well characterized. In particular, systems which remove both phosphorus and nitrogen have not been sufficiently studied.

While many researchers agree that poly-P bacteria are capable of performing denitrification (Comeau et al. (1987); Pokethitiyook (1990); Jespersen and Henze (1993); Vlekke (1988); and Kuba (1993)) , further research is needed to ascertain the factors which affect this process. The purpose of this research was to identify those factors by investigating the effect of temperature changes on the denitrification process in a biological nutrient removal system. The specific objectives of this research project were to (i) investigate the effects of temperature on the denitrification kinetics of a bacterial population from a biological nutrient removal system, (ii) investigate the role which PHAs play in the denitrification process, and (iii) determine the effect of temperature on the production and consumption of PHAs.

CHAPTER TWO: LITERATURE REVIEW

Background

Historically, waste treatment has focused on the removal of solids, pathogens, and organic loadings from waste streams. In the past 10 to 20 years, however, significant attention has been focused on the removal of nutrients from waste streams. The two nutrients receiving the most attention are nitrogen and phosphorus. In most freshwater aquatic environments, these nutrients limit the growth of aquatic flora and fauna. Excessive loadings of these nutrients have been known to cause algal blooms and the eutrophication of water bodies.

The most effective way to prevent the degradation of a water body is to control and limit the amount of nutrients that enter it. There are several methods used to control nutrient loadings from point sources such as wastewater treatment plants. The methods used to remove nitrogen from waste streams include breakpoint chlorination, chemical precipitation, air stripping, and biological nitrification/denitrification. Phosphorus control is generally achieved through chemical precipitation or biological phosphorus removal. When simultaneous removal of nitrogen and phosphorus is desired, biological treatment is often considered the most environmentally cost-effective alternative.

Biological removal of nitrogen is achieved through an activated sludge system which alternates aerobic and anoxic zones. When exposed to aerobic conditions, ammonia (NH_3) is oxidized to nitrate (NO_3) by an autotrophic group of bacteria known collectively as nitrifiers. While exposed to anoxic conditions, denitrifying heterotrophic bacteria convert nitrate to elemental nitrogen (N_2), which is ultimately lost to the atmosphere.

Biological phosphorus removal is achieved through an activated sludge system that alternates between anaerobic and aerobic conditions. In the anaerobic zone, phosphorus removing bacteria (poly-P bacteria) release phosphorus to the mixed liquor solution. This phosphorus is stored internally as poly-phosphate granules. When subjected to anaerobic conditions, these granules are used to provide energy for the uptake of volatile fatty acids (VFAs). The VFAs are stored internally as poly-beta-hydroxyalkanoates (PHAs). The two most commonly found PHAs are poly-beta-hydroxybutyrate (PHB) and poly-beta-hydroxyvalerate(PHV). Their chemical formulae are:

CH_3 (on CH), O (on C, double bond):
$-(CH\text{-}CH_2\text{-}C\text{-}O)_n-$

(PHB)

CH_2CH_3 (on CH), O (on C, double bond):
$-(CH\text{-}CH_2\text{-}C\text{-}O)_n-$

(PHV)

When subjected to aerobic conditions, the bacteria consume the internally stored substrate (PHAs) in order to uptake phosphorus from the solution. The net phosphorus uptake is in excess of what was released in the anaerobic stage, resulting in an overall decrease of phosphorus in the solution. Several different genera of bacteria have been identified that are capable of enhanced biological phosphorus removal. These include *Pseudomonas, Acinetobacter, Aeromonas, and Coliforms* (Kavanaugh, 1993).

It has been found that the anaerobic reactor in a biological phosphorus removing system is necessary for the production of VFAs through fermentation (Brodisch, 1985). These volatile fatty acids are required by the poly-P bacteria in order to form PHAs, which are later used to remove phosphorus from solution. During proper operation, as much as 90 percent of the readily biodegradable COD (RBCOD) is sequestered by poly-P organisms as PHAs in the anaerobic reactor (Randall, 1996). The production of PHAs can also occur under aerobic and anoxic conditions as well as anaerobic conditions, provided there are sufficient VFAs present (Gujer, 1995). The presence of VFAs in the aerobic zone has been found to inhibit the uptake of phosphorus (Wentzel, 1988) since poly-P bacteria will produce PHAs under these conditions rather than utilize them for the uptake of phosphorus. Furthermore, the presence of nitrates in the anaerobic zone has been shown to inhibit biological phosphorus removal by providing an electron acceptor for the non-poly-P bacteria. These bacteria then consume much of the RBCOD in solution, preventing the poly-P bacteria from sequestering substrate as PHAs. This decrease in internal substrate leads to a decrease in phosphorus removal from the system.

Temp effects on denitrification

Over the past few decades, a great deal of research has been conducted to determine the effects of temperature upon biological nutrient removal, particularly nitrogen removal. It is necessary to determine the performance characteristics of these systems as a function of temperature in order to effectively utilize these systems under different operating conditions. The relationship between temperature and denitrification rate for a suspended growth system (not a phosphorus removing system) has been shown to be bell shaped, with a minimum near 0 degress Celsius (°C), and a maximum near 40 °C (Christensen, 1977).

The Arrhenius equation has been used to describe the effect of temperature upon bacterial growth rates. This equation is

$$k_t = A\, e^{-E/RT}, \text{ where}$$

k_t = the kinetic rate of the reaction
A = the frequency factor
E = the activation energy
R = the universal gas constant
T = absolute temperature

The kinetics of bacterial denitrification have also been described theoretically using this equation (Halmo, 1981; Christensen, 1977).

Halmo (1981) found that low temperature denitrification was possible, when psychrophilic denitrifiers were selected. In fact, at low temperatures (0 - 17 °C), these bacteria denitrified at a much higher rate than the mesophilic bacteria (1.5 - 4 times). The relationship between denitrification rate and temperature for these psychrophilic bacteria also follow a bell shaped curve, with a maximum denitrification rate at 12.5 °C. Above this temperature, the performance of these bacteria decreased. The mesophilic bacteria, however, showed a continual increase in denitrification rate from the lowest (0 °C) temperatures to the highest (25 °C). These data are shown in figure 1. If the relationship between denitrification rate and temperature were studied at temperatures greater than 25 degrees, the same bell shaped curve would have represented the mesophilic bacteria. The substrate used for these tests was methanol, and the psychrophilic bacteria were obtained from the hypolimnion of a lake.

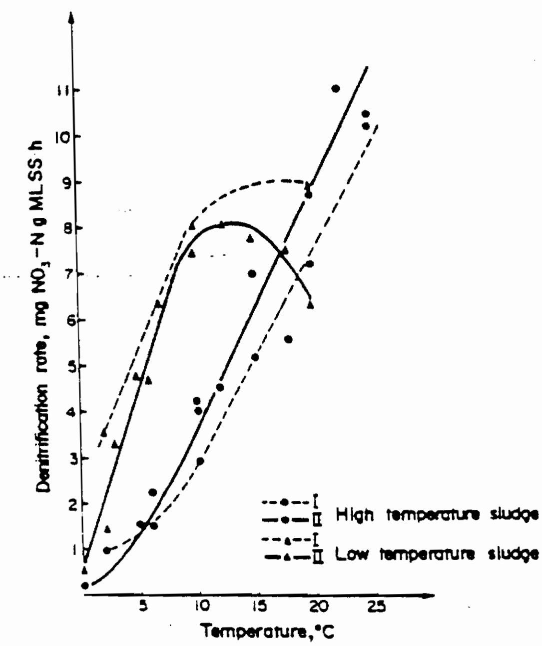

Figure 1. Specific denitrification rate versus temperature for 2 batch tests (from Halmo, 1981). Sludges were enriched at 5 and 20 degrees, respectively. Two different sets of batch tests were performed using different bacterial inocula from the same source.

Previous work by Sutton (1975) and Dawson and Murphy (1972) also found that denitrification at lower temperatures (5 - 25 °C) was possible. In the experiments by Sutton (1975), activated sludge bacteria were fed a combination of municipal wastewater and methanol. The rates obtained from this investigation were lower than those found by Halmo (1981), probably due to differences in both the bacterial culture and the substrate used. The experiments by Dawson and Murphy (1972) obtained much higher denitrification rates than any others, since a pure culture of *Pseudomonas denitrificans* were used.

It has been shown that denitrification at lower temperatures is possible, though rates obtained are lower than those at higher temperatures. The one exception appears to be when psychrophilic bacteria are selected as the primary denitrifiers in the bacterial culture. At temperatures below 25 °C, the relationship between denitrification rate and temperature for activated sludge bacteria appears to be represented by the Arrhenius equation.

Temperature effects upon poly-P systems

Like denitrification, the temperature dependency of biological phosphorus removal is a topic which has been investigated in recent years. According to Sedlak (1991), both Vinconneau and coworkers (1985) and Sell and coworkers (1981) reported that poly-P bacteria removed more phosphorus from solution at 5 °C than at 15 °C. Sell attributes this increase in phosphorus removal to a population shift within the poly-P bacteria to psychrophilic bacteria with a higher yield. Work by von Consbruch (1995) appears to corroborate this statement, as yield values for this phosphorus removing system increased with a decrease in temperature, until about 9 °C, at which point the yield decreased. Results from the research by von Consbruch (1995) appear to indicate an optimum operating temperature for phosphorus removing systems of about 10 °Celsius.

While the optimum operating temperature may be a subject of debate, it is clear that poly-P systems are not as adversely affected by lower temperatures as denitrifying systems. It is unclear, however, what the temperature effects upon denitrification are within a nutrient removal plant that removes both phosphorus and nitrogen.

Debate over poly-P denitrification

The subject of poly-P denitrification has been a highly debated issue in recent years. Wentzel (1989) has reported that enhanced cultures of poly-P bacteria do not denitrify. Other work by Clayton et al. (1989) came to the same conclusion. In the work by Clayton (1989), PHB levels were measured under denitrifying conditions and no significant decrease took place through the first 4 hours. Clayton concluded that SBCOD was being utilized by the bacterial population for denitrification and that poly-P bacteria cannot denitrify.

Since the vast majority of RBCOD is unavailable to the non-poly-P community, a decrease in denitrification rates in a BNR system removing both nitrogen and phosphorus has been hypothesized (Griffiths, 1994). This statement holds as long as the system is operated properly, such that the anaerobic zone does not become anoxic (Hascoet, 1985; Fukase, 1985; and Iwema and Meunier, 1985), and if the assumption that poly-P bacteria are incapable of denitrifying is valid. A substantial decrease in denitrification rate has not always been witnessed, however. In many phosphorus and nitrogen removal systems, nitrogen removal was achieved to the same degree as in systems which did not remove phosphorus. According to Griffiths (1994), studies by both Nicholls & coworkers and Sibritz have concluded that denitrification occurs to the same degree in systems which remove both phosphorus and nitrogen as it does in systems which remove only nitrogen. For this reason, many researchers believe that at least a portion of poly-P bacteria are capable of denitrifying.

Comeau et al. (1987) found that nitrate can serve as an alternate electron acceptor to oxygen for poly-P bacteria, while nitrite cannot. Pokethitiyook (1990) and Jespersen and Henze (1993) also found that nitrate can serve as the final electron acceptor in a biological phosphorus removal system. In these studies, nitrate was used as electron acceptor by the poly-P bacteria to remove phosphorus from solution. In this process, the nitrate was reduced to nitrite. Lotter (1985) performed work on *Acinetobacter* bacteria, one of the predominant species of poly-P bacteria, and found that while a significant portion (30 - 40 %) of *Acinetobacter* were capable of denitrifying to nitrite, very few were able to completely denitrify to N_2. Lotter also found that *Acinetobacter* were capable of producing PHB from both acetate and glucose.

Work by Vlekke et al. (1988) and Kuba (1993) both found that poly-P bacteria were capable of denitrification. Both of these experiments used acclimated samples, suggesting that only certain poly-P bacteria may be capable of this. Comeau et al. (1987) supported this hypothesis, stating that some poly-P bacteria were capable of denitrifying while others were not. Furthermore, Comeau (1987) stated that some of these poly-P bacteria performed the denitrification process using PHAs, while others did not. The group of poly-P bacteria that did not use PHAs may include *Acinetobacter*, which have been found to be capable of using substrate other than PHAs for denitrification (Lotter, 1985; Ante, 1994).

Kavanaugh (1994) reported that a definite shift in bacterial population took place due to the type of substrate being utilized by bacteria. This shift may help account for some of the discrepancies in previous work, since some tests were conducted using wastewater, while others used methanol, acetate, etc. Kavanaugh also reported that several different genera of poly-P bacteria were involved in denitrification (*Acinetobacter, Pseudomonas, Aeromonas*), though most of these bacteria can only denitrify to nitrite. Lotter (1985) supported this finding, stating that *Pseudomonas spp.* were capable of both denitrification and phosphorus removal.

Summary

While the effect of temperature changes on the biological nitrogen removal process have been investigated many times in the past 20 years, the effect of temperature changes on systems which remove both nitrogen and phosphorus has not. In particular, the effect of temperature changes on the denitrification process in these systems needs to be more thoroughly investigated. In recent years, the role which poly-P bacteria play in the nitrogen removal process has received significant attention. This attention has resulted in a debate over whether poly-P bacteria are capable of denitrification. Further research is needed in order to resolve this issue and determine what factors may influence poly-P denitrification.

CHAPTER THREE: MATERIALS AND METHODS

Batch Test Procedure

Mixed liquor for these experiments was obtained from a pilot scale UCT plant located on the Virginia Tech campus. A schematic of the plant is shown in figure 2, while its operating characteristics are listed in table 1.

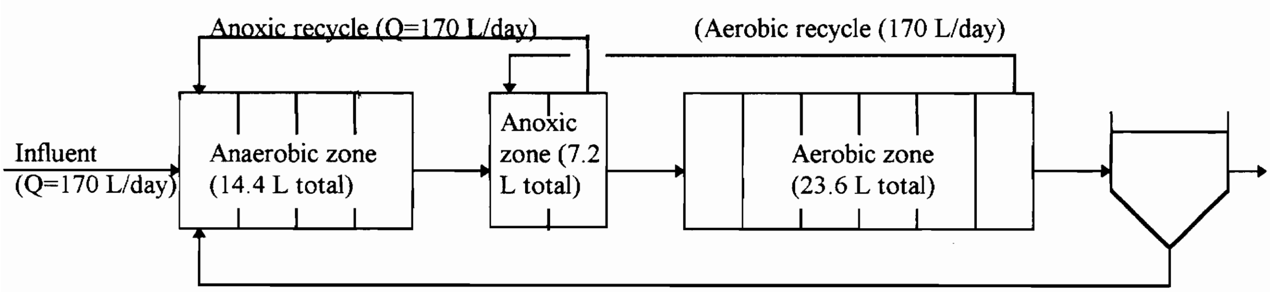

Figure 2. Schematic of the UCT pilot plant.

Sacrificial batch experiments were performed using sludge from the pilot plant in order to assess the kinetic and chemical trends present in the denitrification process.

These batch tests were conducted in 40 mL amber vials at constant temperature. In order to ensure anaerobic conditions, each vial was loosely capped and nitrogen gas was

Table 1. Parameters under which pilot plant was operated.

Parameter	**Value**
MCRT	15 days
HRT	6.4 hours
pH	6.5 - 7.0
Influent COD	320 mg/L
Influent acetate	100 - 170 mg/L
Influent phosphate	20 - 27 mg/L
Influent nitrate	< 0.05 mg/L
Influent magnesium	3.7 mg/L as Mg^{++}
Influent calcium	3.2 mg/L as Ca^{++}
Influent potassium	4.1 mg/L as K^{+}

injected through the septum by a 1.5 inch 21 gauge needle. The nitrogen was used to displace the air that was originally contained in the vial. The displaced air escaped through the space between the threads of the cap and the vial. After purging for at least 15 minutes, the cap was sealed with a Teflon septum and the needle was removed.

Mixed liquor for the batch tests were obtained from the final anaerobic stage of the pilot plant, in order to minimize soluble (readily biodegradable) substrate, nitrate, and dissolved oxygen, and maximize PHB content within the poly-P cells. Solids were removed from the reactor and transferred to the vials using a luer-lok syringe with 1 inch 16 gauge needle to eliminate oxygen entrainment. The caps of the vials were loosened during injection of mixed liquor in order to allow displaced nitrogen gas to escape, preventing a pressure buildup within the vials. Next, the sample vials were placed on a shaker table for 30 minutes to allow the bacteria to consume all remaining COD and dissolved oxygen.

Following this, samples were removed and injected with nitrate, nutrients, and substrate (where applicable). The concentrations of each chemical/ion present in the vials following injection are listed in table 2. Micronutrients were not added, since they would be available in the influent wastewater. After injection, the vials were again placed on the shaker table and were agitated until removed for analysis. When the vials were sacrificed, they were emptied into 15-mL centrifuge tubes (2 centrifuge tubes per vial) and centrifuged at 10,000 rpm for approximately 10 minutes. The supernatant was removed using a syringe and filtered using 0.45 μm pore size membrane filters into 20 mL scintillation vials. These vials were then sealed and frozen until they were ready to be analyzed for nitrates, phosphates, glucose and acetate. The solids in the centrifuge tubes were dried at 105 °Celsius for 24 hours and stored for subsequent PHA analysis.

Table 2. Initial concentrations of chemicals in batch test vials (calculated).

Constituent	Concentration (mg/L)
Nitrate-N	13.9 mg/L[1] as N
Magnesium	3.7 mg/L as Mg^{++}
Calcium	3.2 mg/L as Ca^{++}
Potassium	4.1 mg/L as K^{+}
Ammonium	3.0 mg/L as NH_4^{+}
Acetate (in acetate assays only)	83 mg/L[2]
Glucose (in glucose assays only)	90 mg/L[2]

[1] 15° batch tests used slightly different starting nitrate concentrations.
[2] acetate and glucose concentrations are identical when expressed as COD

Slowly biodegradable COD denitrification rate test

For the slowly biodegradable rate test, duplicate samples were prepared in the same manner as samples in the previous batch tests. Tests were begun following the 30 minute period of RBCOD consumption (on shaker table). At this time, vials were injected with nitrate and nutrients, and vials were sacrificed periodically. Samples were centrifuged and filtered as previously outlined. PHB analysis was not performed on these samples. The mixed liquor for this test was obtained from the pilot plant at 8 °C and the test was run at 20 °C.

Endogenous batch test #1

Duplicate 30 mL samples were withdrawn from the last anaerobic reactor and injected into 40 mL pre-purged vials. These vials were then left shaking for a period of 28 hours before the test was begun in order to allow all COD (readily biodegradable COD and slowly biodegradable COD) to be consumed before the start of the test. Nitrate and macronutrients were then injected into the vials. Vials were subsequently sacrificed over time, and their contents processed as in previous batch tests. Solids were not analyzed for PHB during this test. This test was performed at 10° C.

Endogenous batch test #2

This test was conducted in the same manner as endogenous test 1, except for PHB analysis. For PHB analysis, solids were placed in a 100° C oven within 15 minutes of taking samples.

Endogenous batch test #3 (endogenous rate test)

This test was conducted in the same manner as endogenous batch test #1. PHA analysis was not performed on these samples. This test was conducted at 10 ° C.

PHA Analysis Method

Poly-beta-hydroxybutyrate (PHB) and Poly-beta-hydroxyvalerate (PHV) were both measured using the method described by Hart (1994), with minor variations. The method used for PHA analysis is as follows:

1. 15-30 mL of mixed liquor was centrifuged at 10,000 rpm for at least ten minutes. This volume yielded at least 50 mg of solids, depending upon the mixed liquor concentration.
2. Solids were dried for 24 hours in centrifuge tubes at 105 °Celsius.
3. Solids were removed and weighed as they were placed into a 5 mL high pressure Wheaton "V-vial".
4. At least 5 external standards were prepared in the same manner, with varying PHB and PHV masses. These masses ranged from 0 mg to 15 mg, since this

yielded PHB and PHV concentrations similar to those found in the actual samples.

5. A 3% sulfuric acid in methanol solution (v/v) was prepared.

6. 1.695 g sodium benzoate was added to 100 mL methanol.

7. 2 mL of the sulfuric acid solution was pipetted into each vial using an Eppendorf pipette.

8. 100 μL of the sodium benzoate solution was added to each vial, forming an internal standard.

9. 2 mL of chloroform were added to each vial using an Eppendorf pipette. The vials were then sealed by tightening the caps.

10. Vials were then incubated in the oven at 105 °Celsius for 3.5 hours, at which time the vials were removed and allowed to cool.

11. 1 mL distilled water was then added to each of the vials, which were then resealed and placed on a shaker table for 10 minutes to allow adequate mixing of the organic and inorganic layers. Upon sitting, the chloroform would settle and the water/methanol would rise.

12. Upon completion of this step, vials may be stored for up to 3 weeks in a Flammable Materials refrigerator. When GC analysis was required, the vials were allowed to warm up to room temperature before injection.

13. 1 μL of the chloroform phase (bottom layer) was injected into a 3 meter 2% Reoplex 400 Chromosorb GAW column attached to an FID (Flame Ionization Detector). A Hewlett Packard 5880 A Gas Chromatograph was used for the analysis.

14. Using the GC settings in Table 3, chloroform appeared as several peaks covering the first minute of run time. PHB then appeared as a peak at about 3.5 minutes, PHV appeared at about 5 minutes, and sodium benzoate appeared at 6.5 minutes.

Table 3. Gas Chromatograph Operating Parameters

Parameter	Setting
Oven Temperature	130° C
Injector Temp	160° C
Detector Temp	200° C
H_2 gas pressure	48 psi
Air pressure	38 psi
N_2 gas pressure	48 psi
Attenuation	2↑6
Threshold	4
Peak Width	0.04

PHA results were expressed as a percentage of the total suspended solids concentration (% TSS = mg PHA/mg TSS). This value was calculated by dividing the area of the GC integration for a given sample by the slope of the regression of the external (PHA) standard. This value was then divided by the mass of the sample used (mg) and multiplied by 100 in order to obtain a percentage.

There were several variations from the original method by Hart and Seyfried (1994). These included:

1. Sodium benzoate was used as a standard rather than benzoic acid, since it was desired to keep the standard separate from the rest of the methanol/sulfuric acid solution, to avoid degradation of the standard over time. The method used by Hart and Seyfried used 1 mg benzoic acid in each vial as an internal standard. In order to preserve the accuracy of the standard, it was decided to separate the standard from the sulfuric acid/methanol solution.

2. An external PHB/PHV standard was used, rather than just a PHB standard, in order to track the fates of both PHV and PHB in these experiments. The standard was composed of 76 % PHB and 24 % PHV.

3. In the Hart method, 1.25 mL of the chloroform phase was removed from the PHB vials and stored in separate vials for subsequent injection. Samples in this research were kept in the PHB vials and analyzed by removing the 1 μL injection directly from the chloroform layer in the PHB vial, rather than transferring the solution to another vessel for storage.

Blacksburg/Virginia Tech wastewater treatment plant PHB analysis

The integrity of the PHA analysis method was confirmed using Blacksburg/Virginia Tech wastewater treatment facility sludge, which does not demonstrate enhanced phosphorus removal, as a blank. For this test, sludge was taken from the aerated activated sludge basin of the Blacksburg/VPI Wastewater Treatment Facility, a system which did not exhibit biological phosphorus removal. This sludge was injected with acetate and allowed to react on a shaker table for one hour in order to consume the oxygen in the vessel. After this time, mixed liquor was removed and injected into pre-purged vials. Next, acetate, phosphate, and nutrients were added to the mixed liquor, and the vials were replaced on the shaker table. Triplicate samples were removed at 1 hour, 1.75 hours, and 2.5 hours using methods outlined in the batch test procedure. This test was conducted at 20 degrees.

Nitrate and Phosphate

Nitrate and phosphate levels were measured using ion chromatography method 4110 B, as outlined in Standard Methods (1992). Samples were filtered through 0.45 micron pore size filters before injection into a Dionex 2010I ion chromatograph.

Glucose

Glucose was analyzed using the glucose hexokinase method. Minor modifications were made in order to improve detection. Samples were diluted before analysis to between 1 and 10 mg/L. Assays consisted of 2.4 mL hexpkinase reagent and 1.6 mL diluted sample. Assays were mixed by inversion and analyzed using a fluorescence spectrophotometer. Excitation and emission wavelengths were 344 nm and 457 nm, respectively.

Acetate

Acetate levels were measured using the following process. Samples were centrifuged and filtered through a 0.45 μm membrane. Five mL of filtered sample was placed in a beaker. Twenty five μL of formic acid were added to the beaker. One μL of this solution was then injected into a Tracor 560 Gas Chromatograph. A 0.3 % Carbiwax 20 m column was used for analysis. Gas chromatograph settings are shown in table 4.

Table 4. Gas Chromatograph settings for acetate analysis.

Parameter	Setting
Oven Temperature	120° C
Injector Temp	200° C
Detector Temp	200° C
H_2 gas setting	30
Air setting	0.1
N_2 gas pressure	50 psi
Attenuation	2↑3
Threshold	0
Peak Width	0.04

TSS/VSS analysis

Total suspended solids (TSS) and mixed liquor volatile suspended solids (MLVSS) analyses were performed according to Standard Methods (1992) procedures 2540 D and E.

Chemical Oxygen Demand

Chemical Oxygen Demand was measured according to the closed reflux, titrimetric method, procedure 5220 C in Standard Methods (1992).

CHAPTER FOUR: RESULTS

Results - batch tests

Batch tests were conducted at fifteen °Celsius on July 4 and July 7, after the pilot plant had achieved a steady state for at least 1 month (2 mean cell residence times). Steady state was defined as maintaining relatively constant temperature and mixed liquor volatile suspended solids concentration for at least two MCRTs. The initial batch test (7/4/95) was conducted for two hours. Since the nitrate concentrations in this test did not reach zero, it was decided to allow the remaining batch tests to run for three hours. The results of the 15 degree tests are illustrated in figures 3 and 4. The normalized (to g MLVSS) rates of denitrification for these two tests are shown in Table 5. In these tests, the highest denitrification rates were obtained using glucose as substrate. The unamended samples exhibited the lowest nitrate removal rates.

Table 5. Normalized rates of denitrification-15 °C.
($mg\ NO_3$-N removed/hr/g MLVSS)

	Acetate	**Glucose**	**No Substrate**
Batch 7/4/95	1.63	2.10	1.12
Batch 7/7/95	1.67	2.17	1.59

Three batch tests were conducted at ten °Celsius. These tests were performed on August 8, 9, and 10. The results of these tests are shown in figures 5-7. The normalized denitrification rates are listed in table 6. At ten degrees, the average nitrate removal rates obtained using glucose as substrate were slightly higher than those

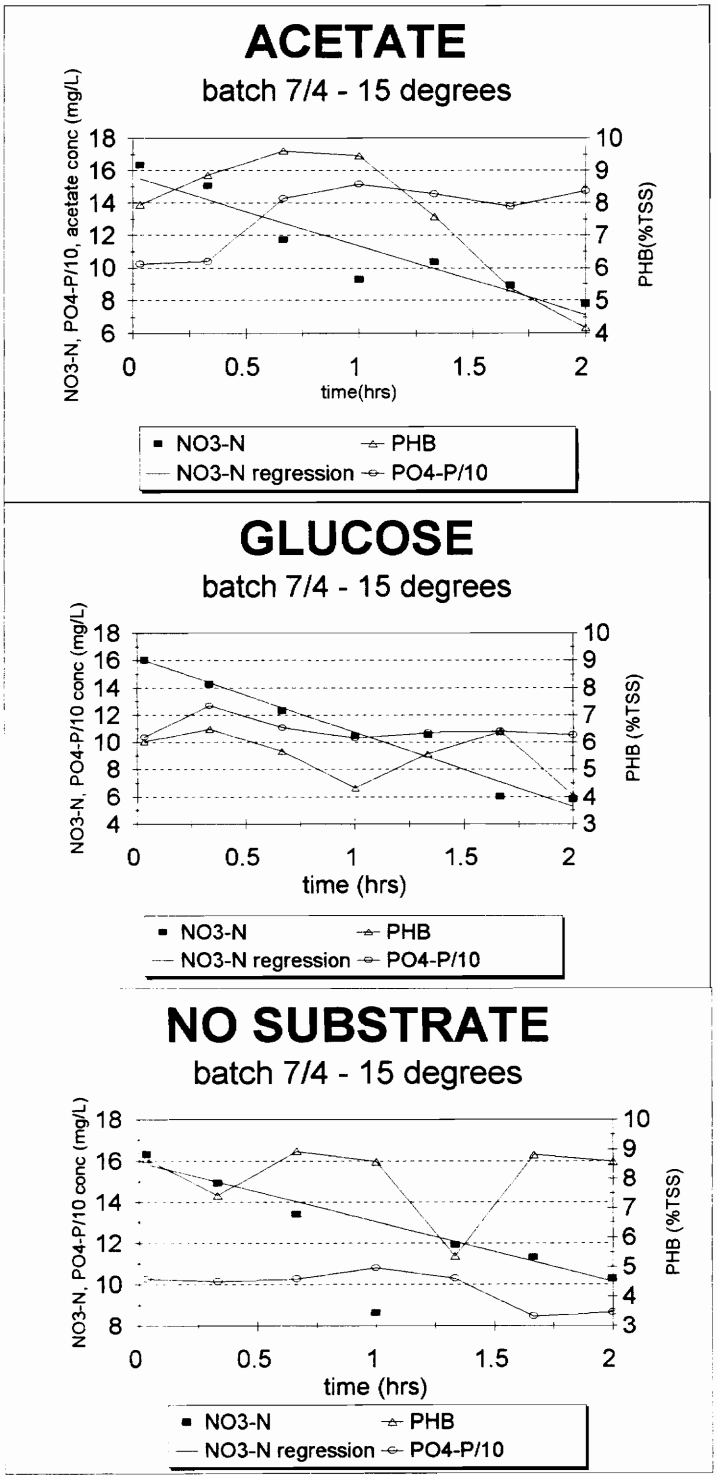

Figure 3. Results from first 15 degree Celsius batch test - 7/4/95.

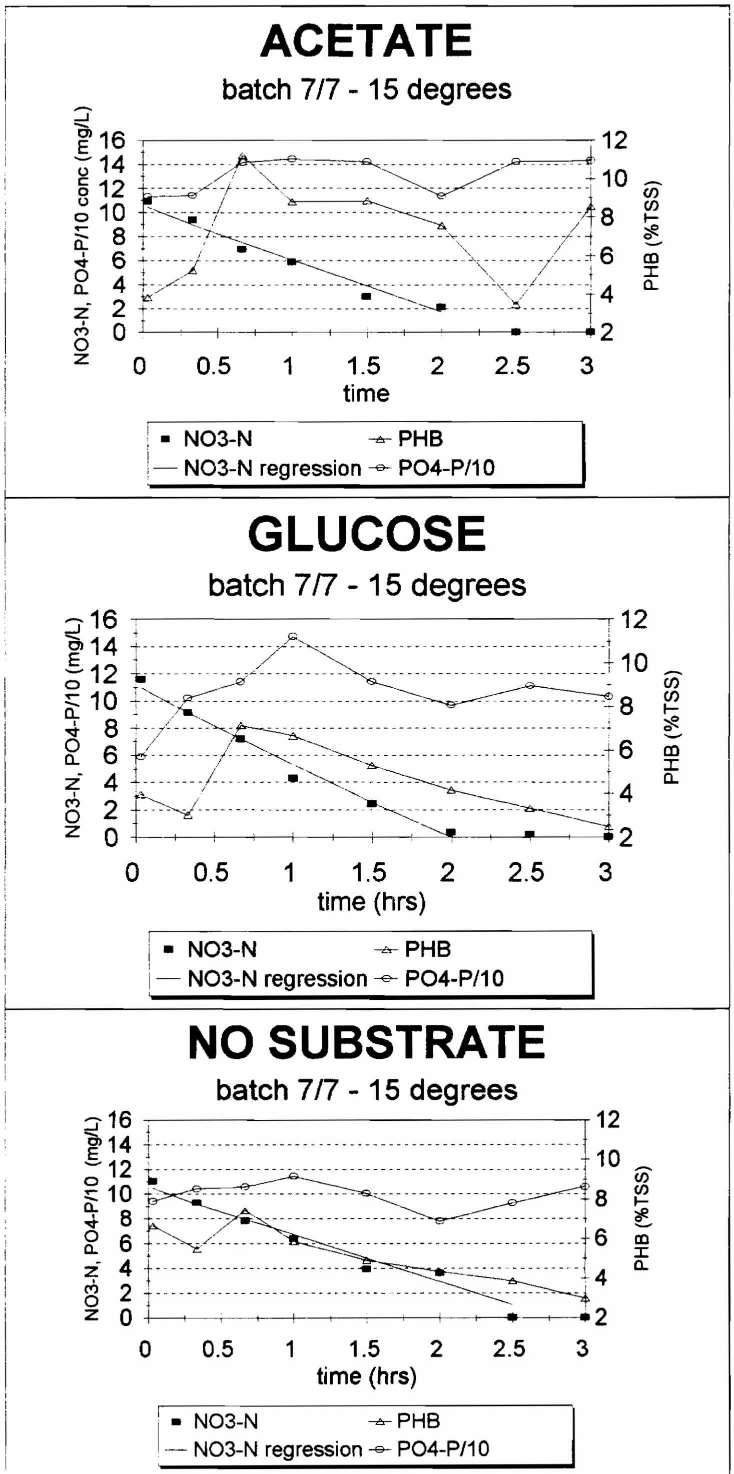

Figure 4. Results from second 15 degree Celsius batch test - 7/7/95

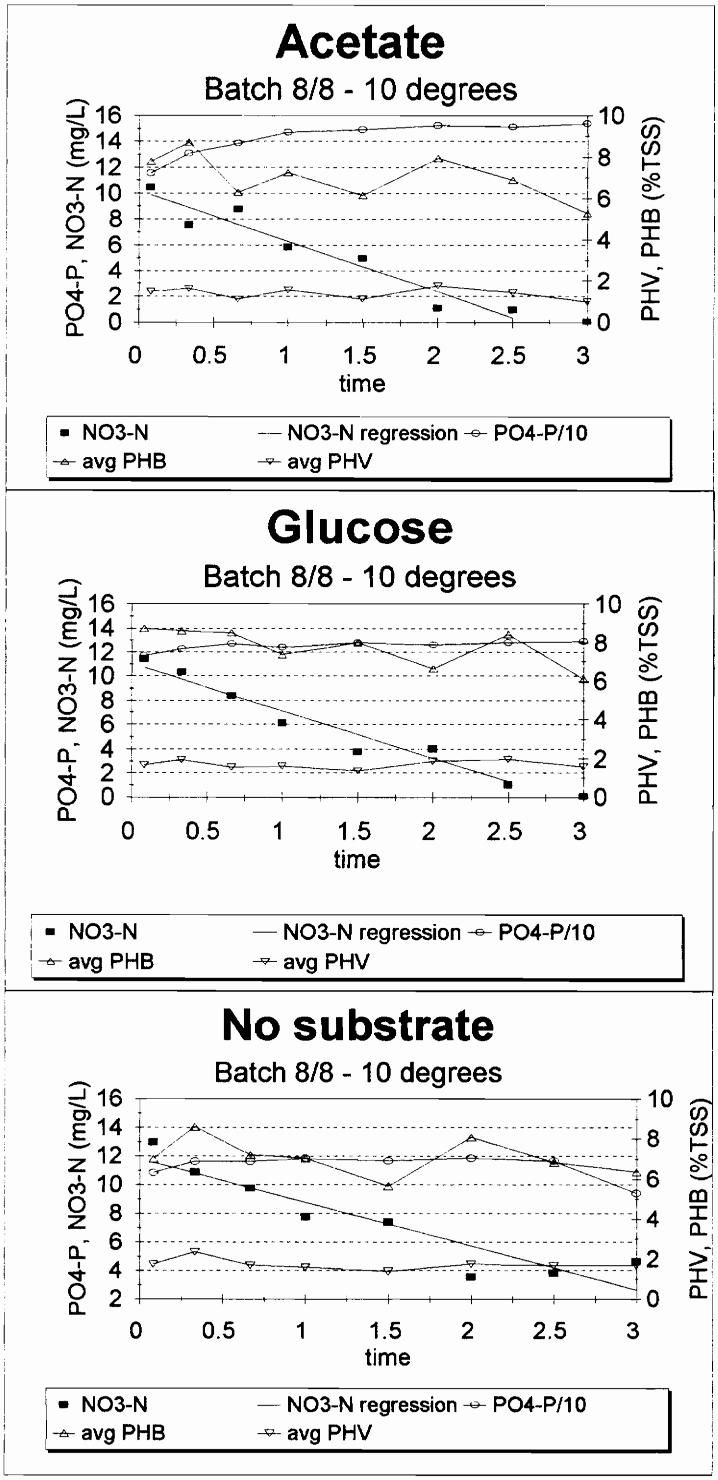

Figure 5. Results from first 10 degree Celsius batch test - 8/8/95.

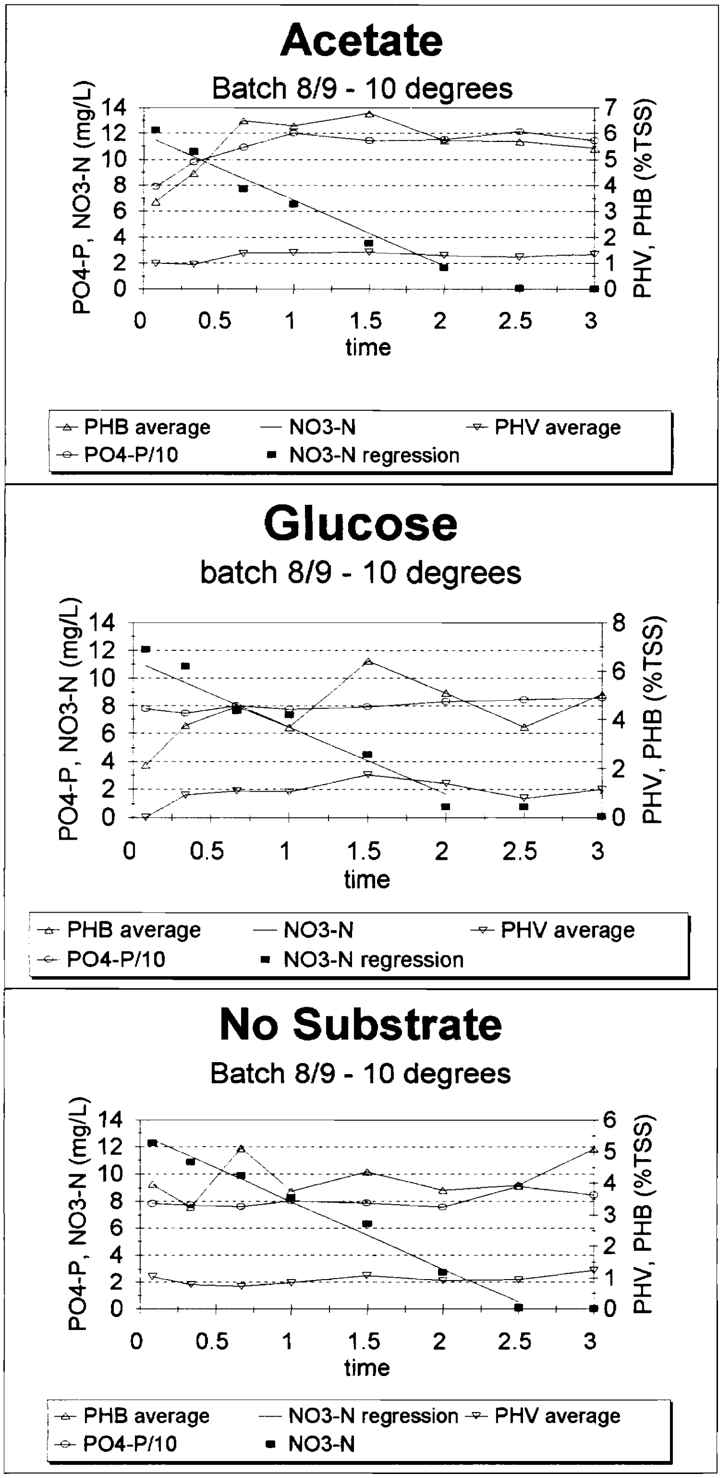

Figure 6. Results from second 10 degree Celsius batch test - 8/9/95.

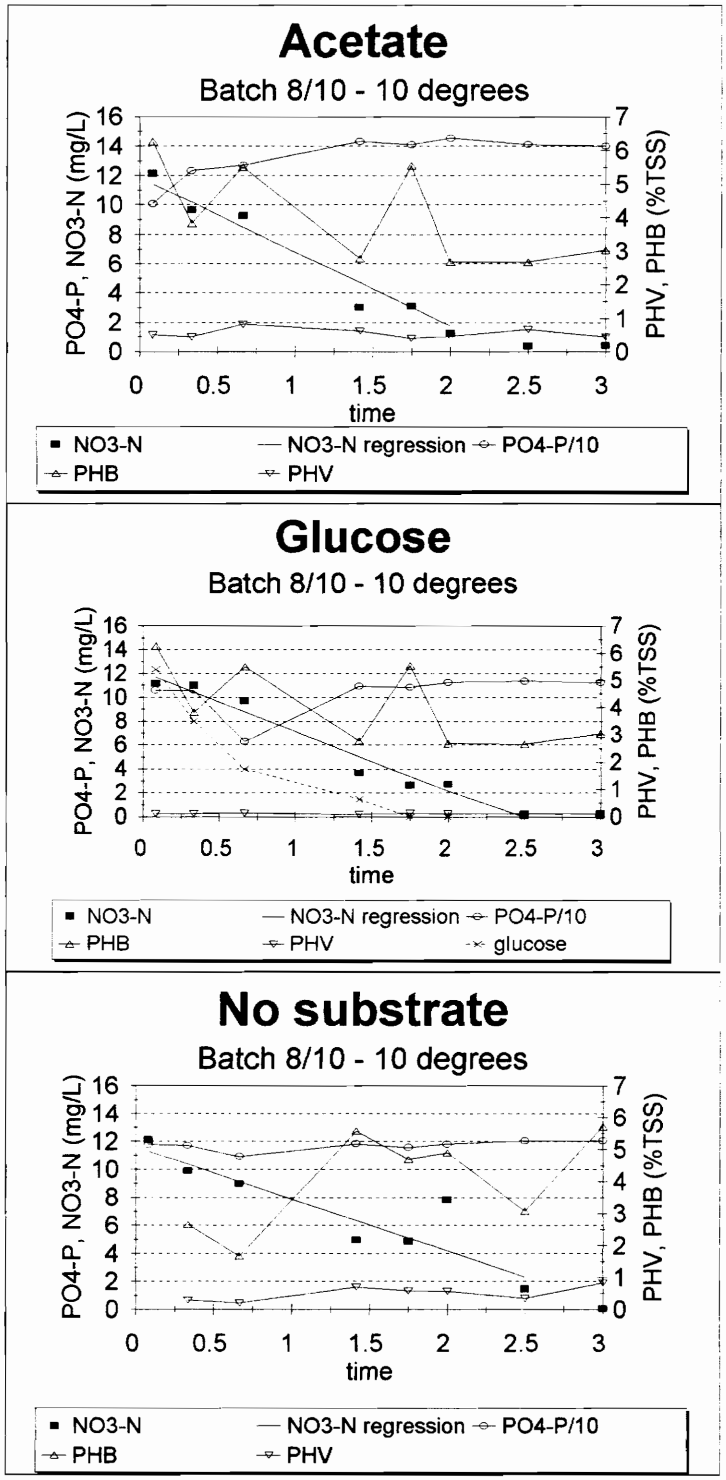

Figure 7. Results from third 10 degree Celsius batch test - 8/10/95.

obtained using acetate as substrate, while the unamended samples continued to exhibit significantly lower denitrification rates.

Table 6. Normalized rates of denitrification-10 °C.
(mg NO_3-N removed/hr/g MLVSS)

	Acetate	Glucose	No Substrate
Batch 8/8/95	1.31	1.30	1.02
Batch 8/9/95	1.69	1.89	1.66
Batch 8/10/95	1.68	1.66	1.24

Batch tests were conducted at 8 °C on October 2, 3, and 4, after the system had been allowed to equilibrate at 8 degrees for 1 month. The results from these batch tests are shown in figures 8, 9, and 10. The normalized denitrification rates are shown in the table below. As shown in figures 3 through 10, there appeared to be no phosphorus uptake by the poly-P bacteria in any of the batch tests.

Table 7. Normalized rates of denitrification-8 °C.
(mg NO_3-N removed/hr/g MLVSS)

	Acetate	Glucose	No Substrate
Batch 10/2/95	1.68	1.21	1.24
Batch 10/3/95	1.58	1.32	1.29
Batch 10/4/95	1.69	1.28	1.19

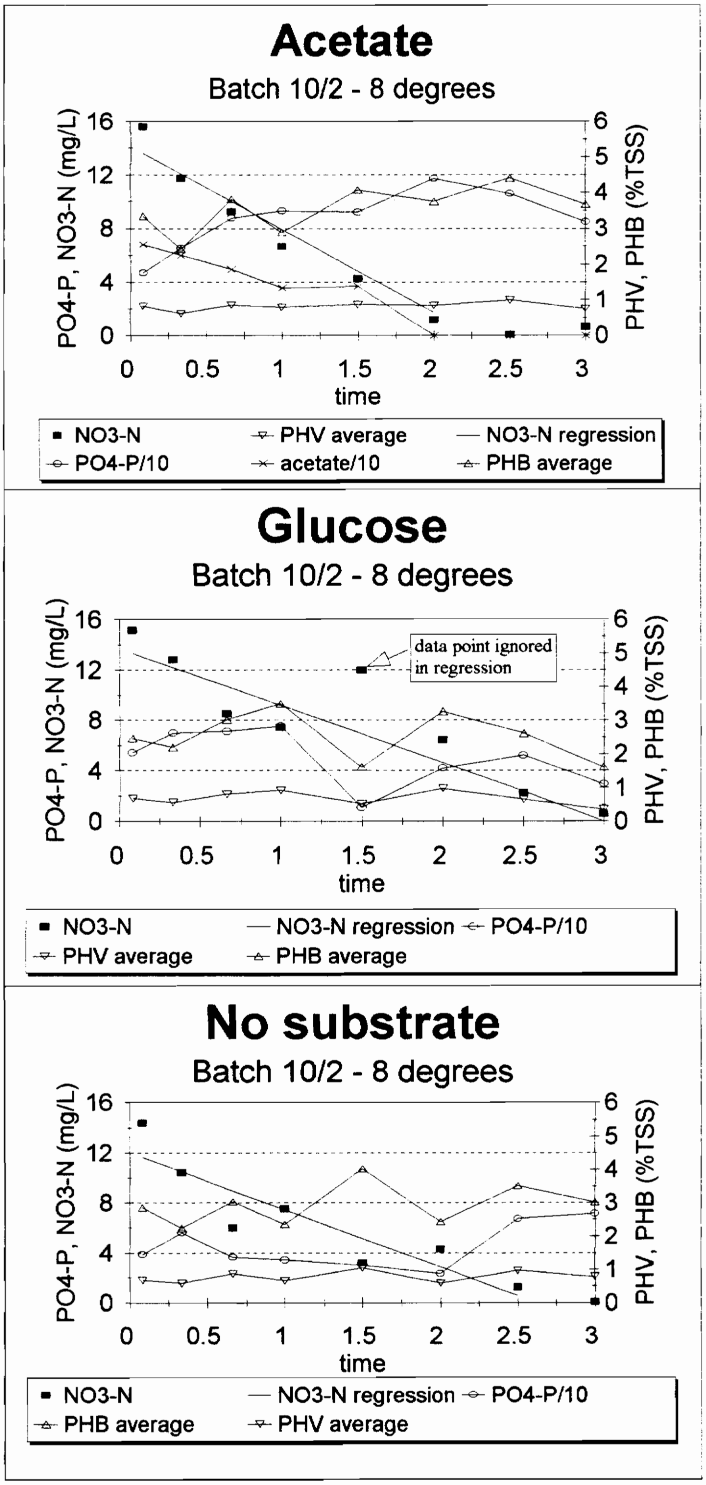

Figure 8. Results from first 8 degree Celsius batch test - 10/2/95.

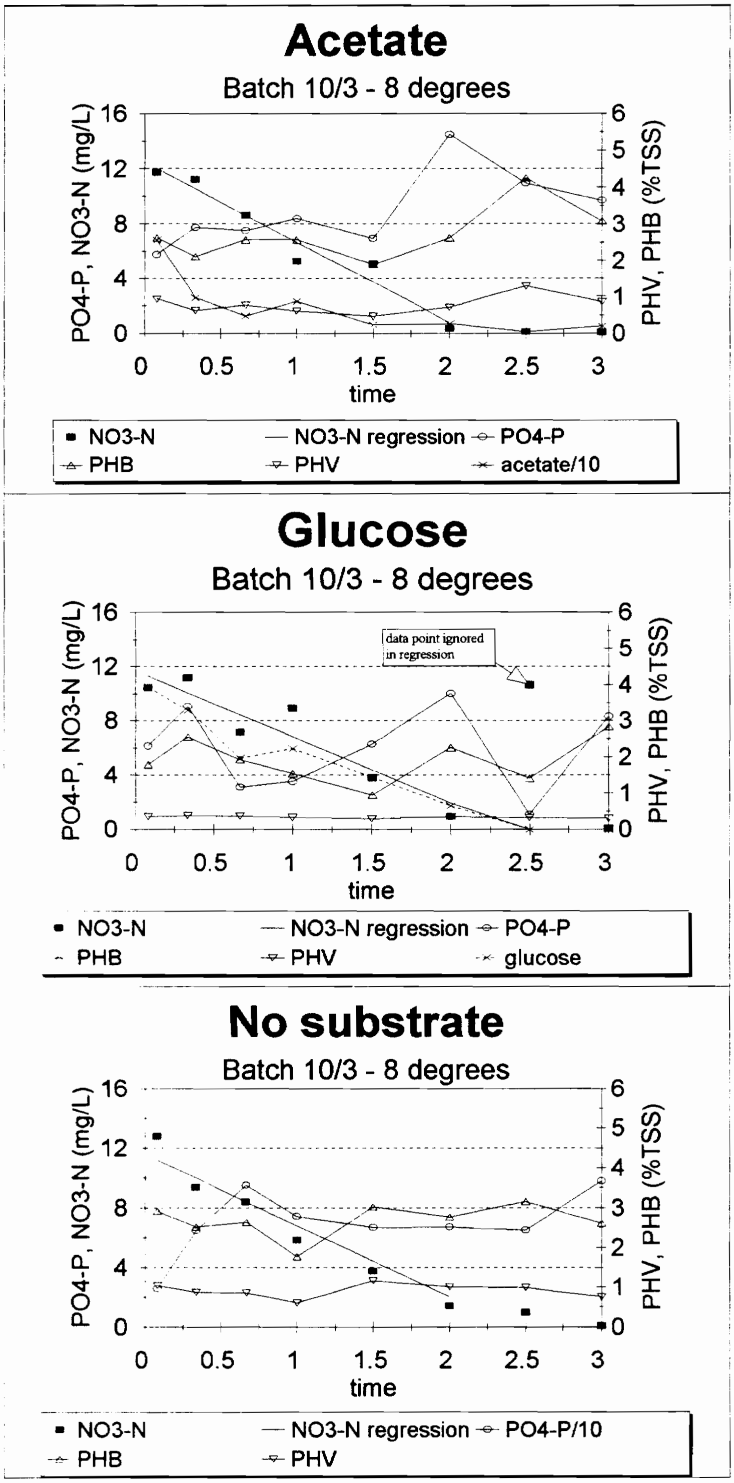

Figure 9. Results from second 8 degree Celsius batch test - 10/3/95

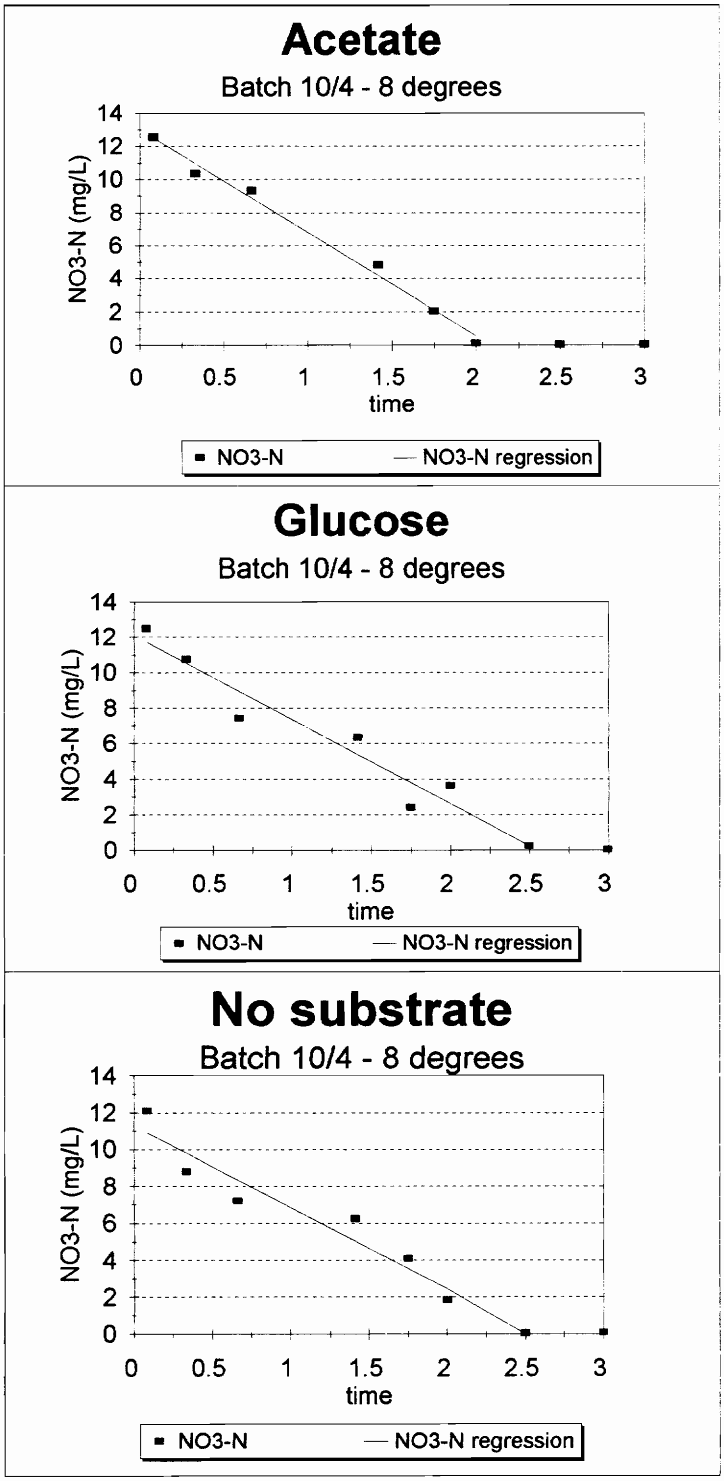

Figure 10. Results from third 8 degree Celsius batch test - 10/4/95.

PHB statistical analyses

Upon completion of the first set of batch tests (15 °C), an effort was made to explore the possibility of using a more accurate PHB analysis method. A second method was found which, according its author (Comeau, et al., 1988), was an improvement of the original method by Braunegg, et al. (1978). The sample preparation for this method was a variation of that used in the original, while the analysis performed by Comeau et al. was done using a capillary column rather than a packed column. A capillary column was used to improve the separation of peaks.

This method involved two changes in the sample preparation. First, samples were lyophilized rather than placed in an oven overnight. The second change involved the removal of most of the chloroform layer following the addition of water. This chloroform solution was then transferred to another vial containing a small quantity of water. This vial was then shaken and centrifuged. This process was intended to purify the chloroform solution by removing particulate matter from the solution, thereby increasing the useful life of the GC column.

Since a capillary column and lyophilizer were not available for this method, the method was modified to accommodate available equipment. Two variations were analyzed. In the first variation, samples were prepared using the same method of re-extraction as outlined above (1.0 mL chloroform phase added to 0.5 mL water, followed by shaking and centrifugation), while a packed column was used, as in the previous PHA analyses. The second variation was identical, except the samples were not shaken. In both tests, samples were dried in an oven overnight. The results of the three PHB statistical tests are shown in table 8 and illustrated in figures 11-13.

Table 8. Results of statistical analyses of various PHB methods.

Method	mean value	95% confidence interval
Method 1 - Hart/Seyfried modified method	10.3	+/- 0.78 % TSS
Method 2 - Comeau modification w/shaking	10.2	+/- 0.95 % TSS[1]
Method 3 - Comeau modification w/o shaking	8.6	+/- 0.93 % TSS

[1] In this test, one of the data points was discarded, since it appeared to be due to an error in the analysis procedure. Otherwise, the value for the 95 % confidence interval would be +/- 1.62 % TSS. For complete results, see Appendix.

As shown in Table 8, the original PHB analysis method as outlined by Hart and Seyfried was the most precise of the methods analyzed. While the complete Comeau method may have been more precise, the proper equipment was unavailable to fully replicate this method. Therefore, further PHB analysis was performed using the method used by Hart (1994) and Seyfried (1994). Unfortunately, the routine experimental error of the PHB analysis made it difficult to determine significant changes in PHB concentrations during several experiments.

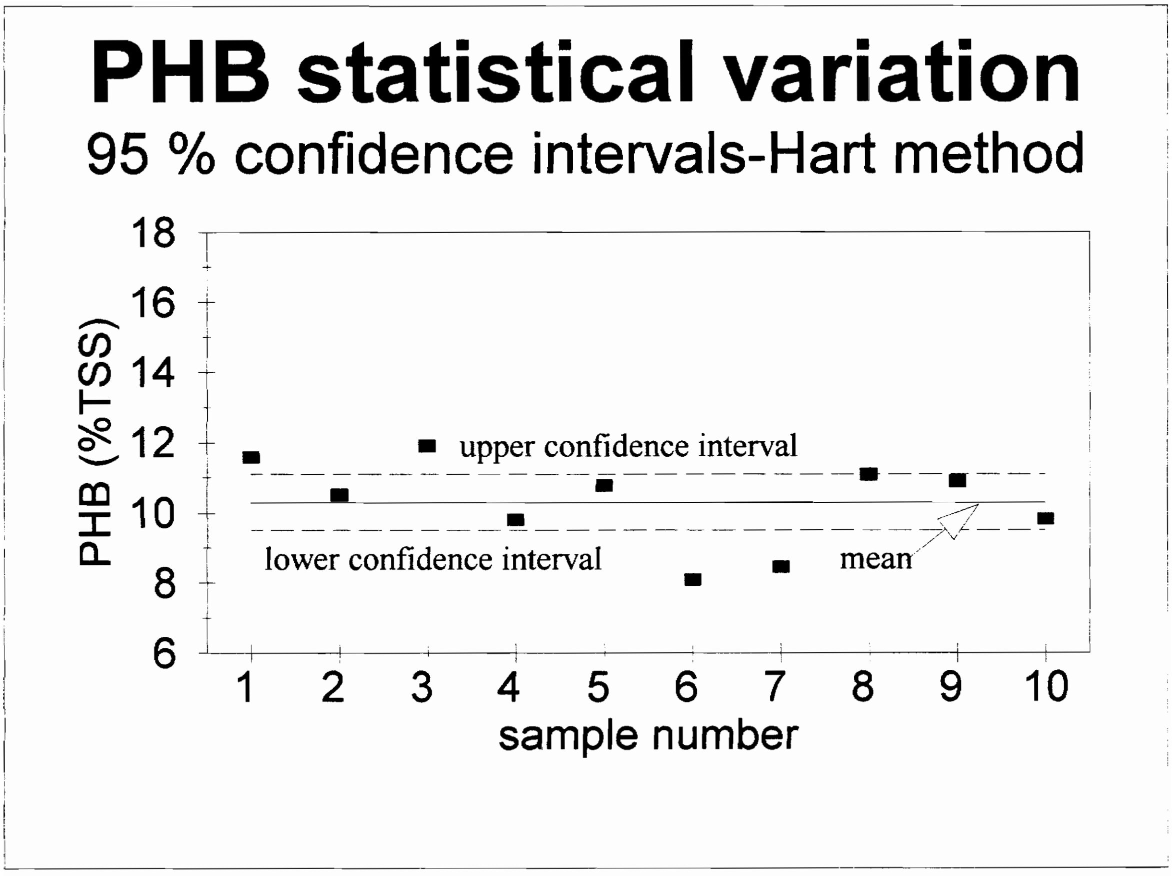

Figure 11. Results from PHB statistical analysis of modified Hart/Seyfried method.

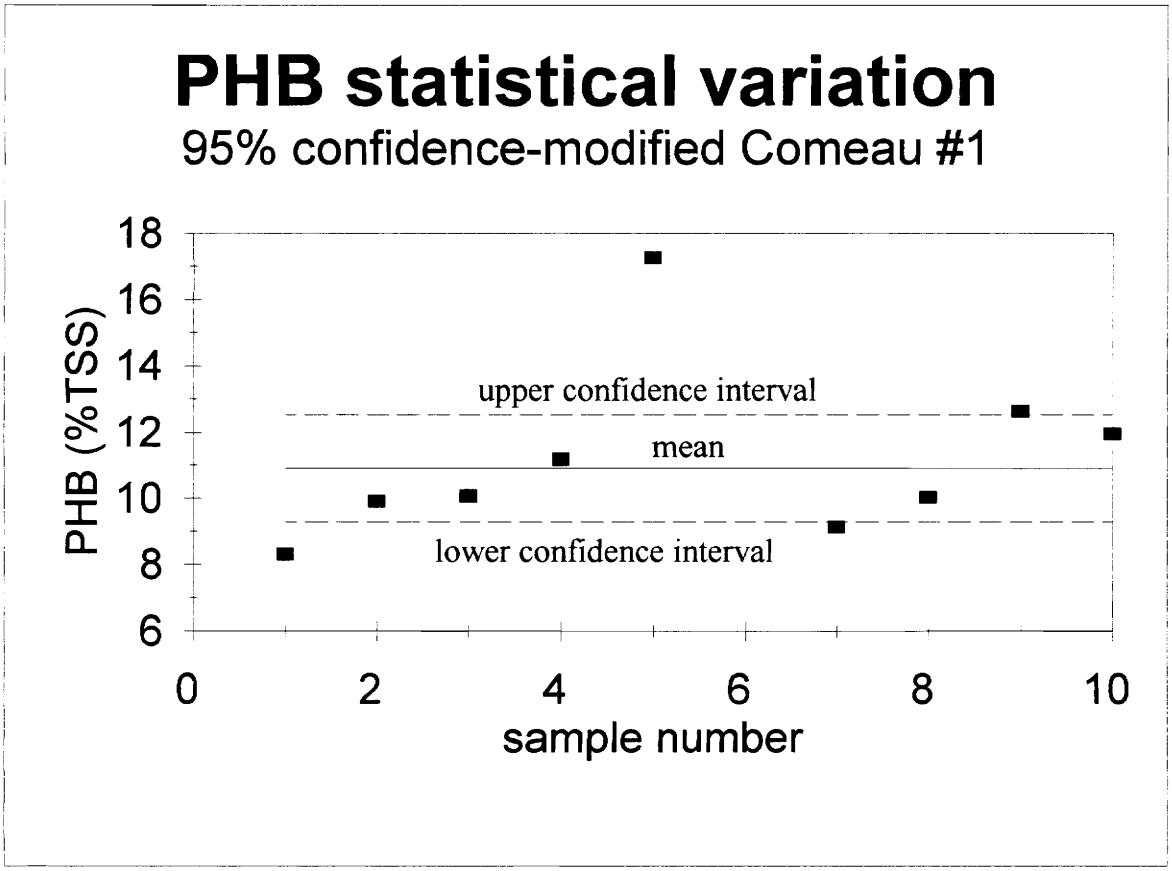

Figure 12. Results from PHB statistical analysis of modified Comeau method # 1.

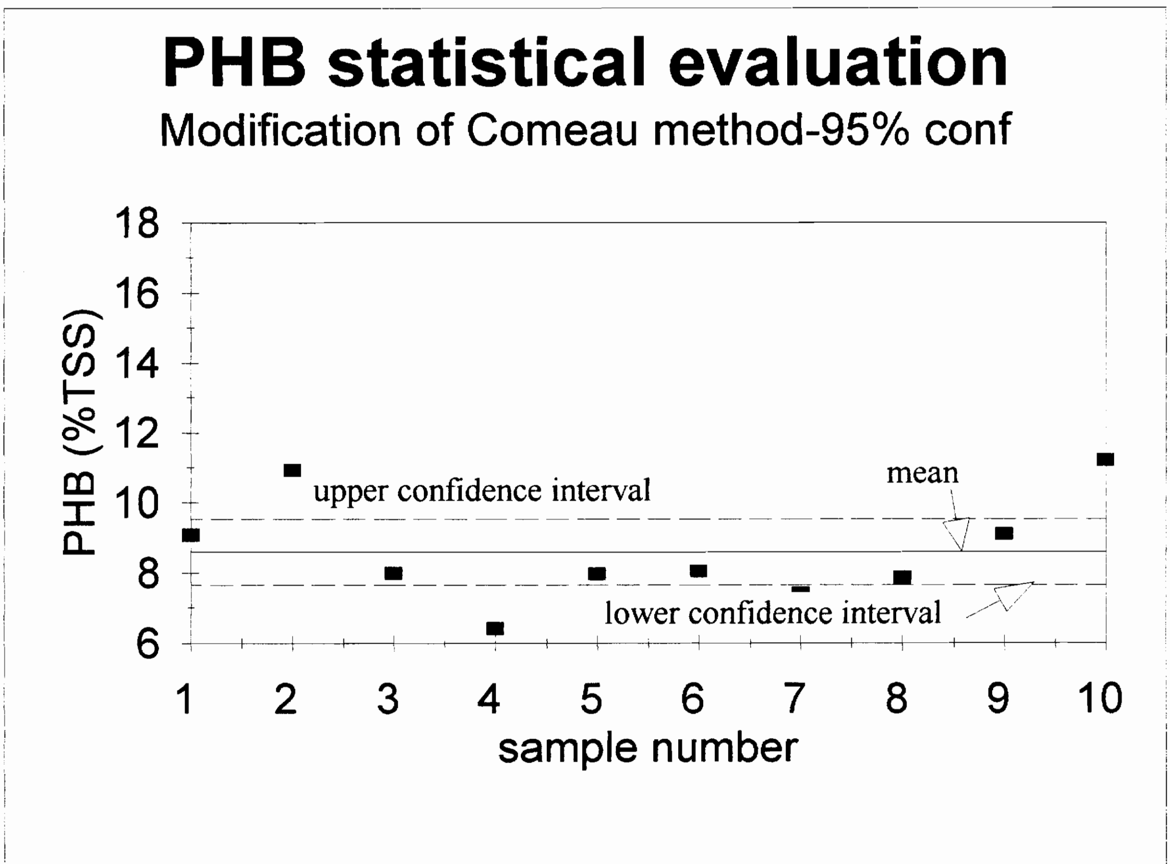

Figure 13. Results from PHB statistical analysis of modified Comeau method # 2.

Blacksburg/Virginia Tech wastewater treatment plant PHB analysis

In order to ensure that PHB levels were indicative of PHB levels in poly-P bacteria, rather than the general bacterial population, it was necessary to quantify background levels of PHB in a sludge that was not exhibiting excessive biological phosphorus removal. This analysis was performed on a biological sample which was intended to represent a blank. For this test, sludge was taken from the aerated activated sludge basin of the Blacksburg/VPI Wastewater Treatment Facility, a system which did not remove phosphorus.

As shown in Table 9, there was very little PHB present in the Blacksburg/VPI. In fact, larger quantities of sludge were used for this PHB analysis than are typically used. Therefore, PHB quantities may not have even been detectable if the typical mass of sludge had been used. Furthermore, the levels of PHB found in this sludge were much lower than the level of error associated with this method (see Table 8). As such, it was assumed that there is little or no PHB found in wastewater treatment systems which do not exhibit biological phosphorus removal. Therefore, PHB levels found in batch tests on the poly-P sludge were solely indicative of levels found in phosphorus removing bacteria.

Table 9. PHB content of Blacksburg/VPI WWTP Sludge.

Time	% TSS
1 hour	0.52
1 hour	0.33
1 hour	0.29
1.75 hours	0.41
1.75 hours	0.61
1.75 hours	0.38
2.5 hours	0.33
2.5 hours	0.49
2.5 hours	0.57

Results - Endogenous and slowly biodegradable rate tests

Four separate tests were performed in an effort to determine what the bacteria in the no substrate tests were using as substrate for denitrification. Three of these tests were endogenous tests while the fourth was a slowly biodegradable COD rate test. The information obtained from these tests was used to determine whether the bacteria in the no substrate samples of the batch tests were denitrifying using SBCOD as substrate, or were using decay products from other bacterial cells as substrate. These tests were also used to help determine whether poly-P bacteria were capable of denitrifying using PHAs as substrate. It was hypothesized that if poly-P bacteria are capable of denitrifying while using PHAs as an electron donor, then they would be most inclined to do so when starved of external electron donors.

The first test was performed at ten degrees and was intended to determine the fate of PHAs in an anoxic system that was under endogenous conditions. PHA samples were taken over a 22 hour time period, while nitrate was analyzed for the first 9 hours. As shown in figure 14, PHA concentrations appeared to decline for the first 6 hours, at which point the decrease in PHA concentrations ceased. After 6 hours, the PHA data were more variable. This variability was not very great, due to the magnitude of the PHA concentrations and the experimental error associated with the PHA method. Due to the inconclusive nature of this test, another similar test was performed.

The second test was also performed in order to determine the fate of PHAs in endogenous denitrification. The results from this experiment are shown in Figure 15. These tests were also performed at a temperature of 10 degrees. As shown in figure 15, it appeared there was a reduction in PHB levels over the time of the experiment, but exactly when this reduction occurred and its extent are unclear. The magnitude of this reduction is clearly greater than the experimental error of the method, however. While there did appear to be a reduction in PHA concentrations within the biomass, PHA concentrations remained significant throughout the test, despite the presence of an electron acceptor in solution. In this test, the specific nitrate removal rate was 0.18 mg NO_3-N removed/hr/g MLVSS. This rate was found to be nearly an order of magnitude lower than other rates obtained at the same temperature and was therefore clearly representative of an endogenous denitrification rate.

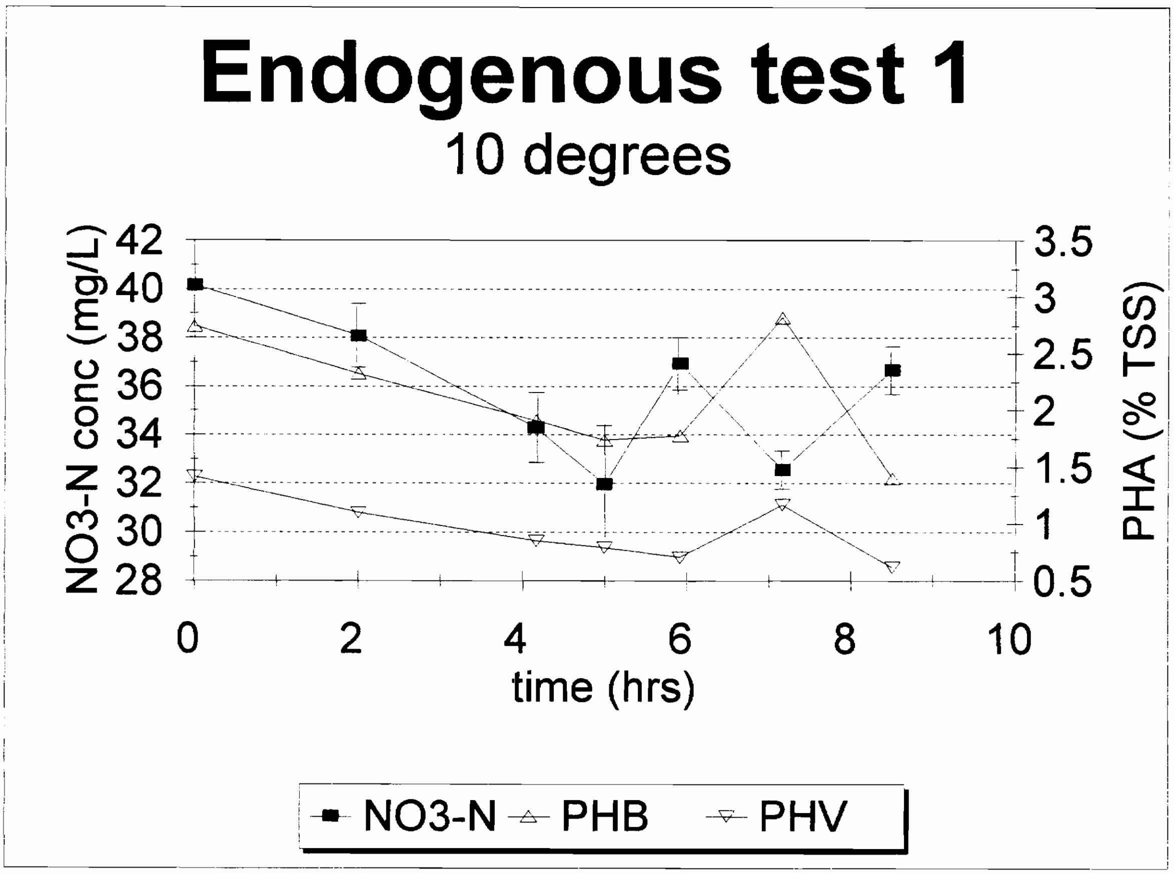

Figure 14. Results from endogenous test # 1.

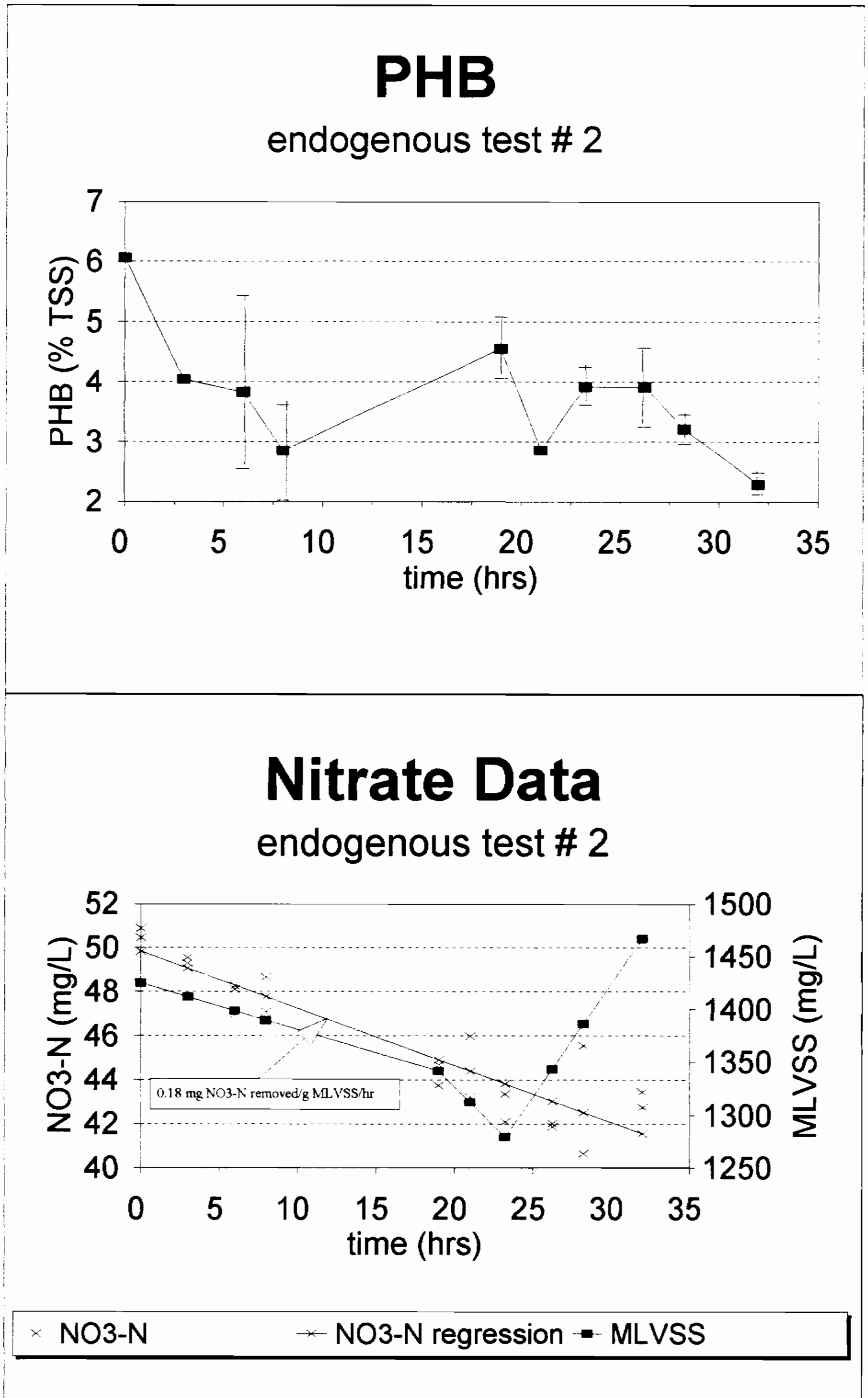

Figure 15. Results from endogenous test # 2.

The third endogenous test was performed in order to determine the endogenous denitrification rate of this particular mixed liquor. The results of this experiment are illustrated in Figure 16. This test yielded a normalized endogenous denitrification rate of 0.57 mg NO_3-N removed/hr/g MLVSS at 10 degrees. This endogenous denitrification rate is considered more accurate than the one mentioned in endogenous test # 2 since the system was at steady state for this test, while it had not quite achieved steady state at the time endogenous test # 2 was performed.

In the SBCOD test, samples were analyzed to determine the rate at which nitrate was removed from solution in the first two hours after nitrate addition. The mixed liquor for the SBCOD test was obtained at 8 °C, and the test was run at an ambient temperature of 20 °C. Therefore, its results were not meant to be considered the SBCOD denitrification rate at any particular temperature, but simply a rate that could be compared loosely to an endogenous rate. If these numbers were found to be significantly different, it would be assumed that they would be different even if the tests were run at the same temperature.

This test produced an SBCOD denitrification rate of 0.9 mg NO_3-N removed/hr/g MLVSS. The results of this test are illustrated in Figure 17. As illustrated, the least squares fit of the data only used the final seven data points, since the others did not appear to be linear. The first three points may have represented an acclimation period, during which time the bacteria were adjusting to denitrifying conditions within the vials.

The SBCOD denitrification rate was slightly lower than the rates obtained in the batch tests, but higher than the measured endogenous rates.

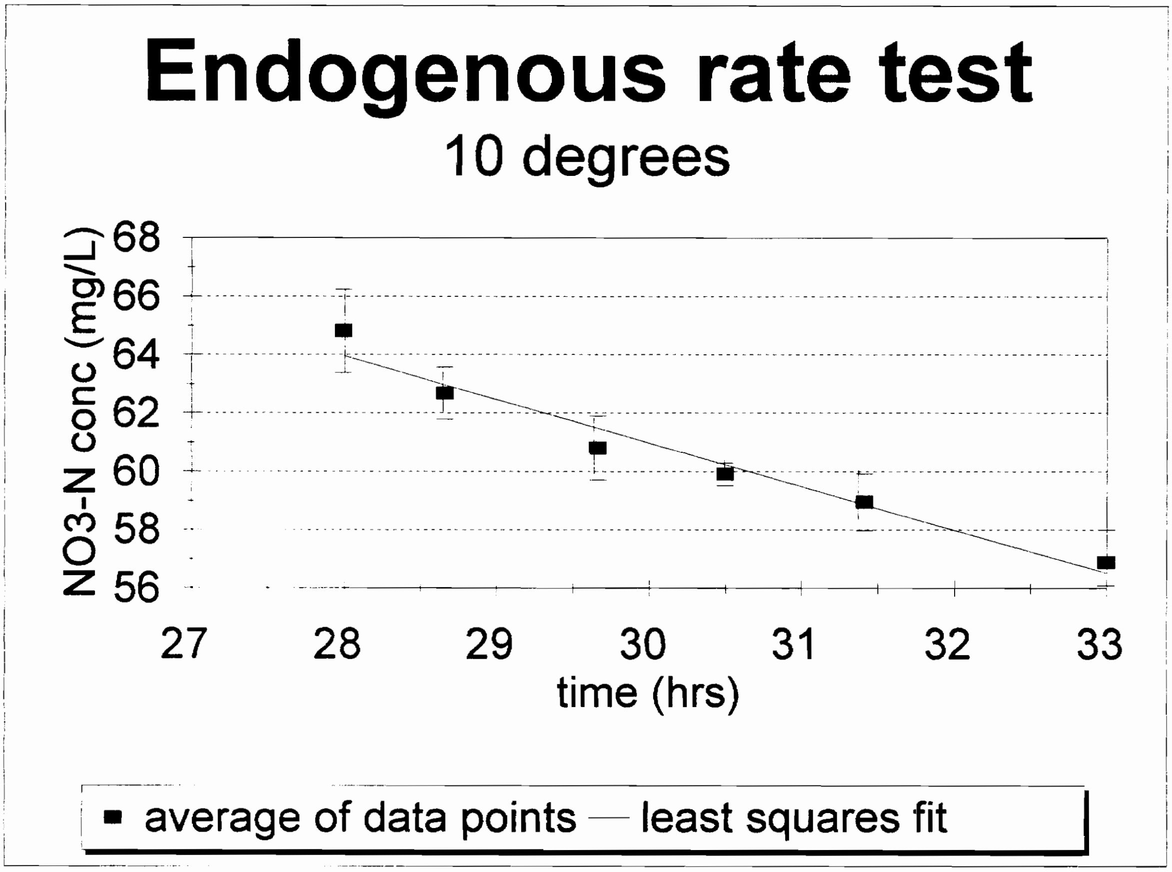

Figure 16. Results from endogenous rate test.

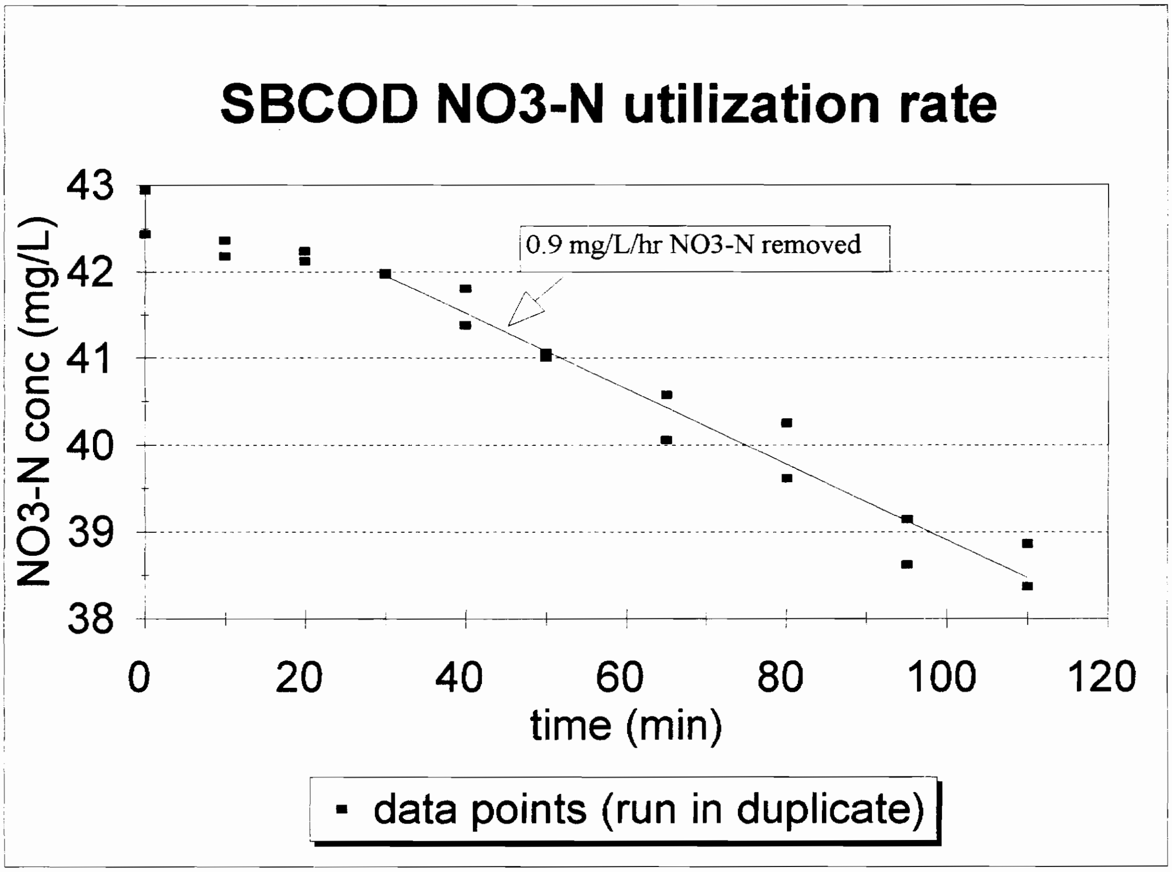

Figure 17. Results from SBCOD test.

CHAPTER FIVE: DISCUSSION

Batch tests

At 15 degrees, it is believed that glucose was being utilized primarily by non-poly-P bacteria, while acetate was used by both poly-P and other bacteria. This conclusion is based upon figure 19. As illustrated, denitrification rates for the glucose amended samples decreased with a decrease in temperature. This same trend has been found numerous times for denitrifiers in an activated sludge system (Sutton, 1975; Stensel, 1970; and Christensen (1977) reported similar findings in both Mulbarger and Johnson & Vania). Furthermore, these denitrifiers would have an advantage over poly-P bacteria in using glucose, since most poly-P bacteria would have to break the glucose down into volatile fatty acids before being able to use it as substrate, while the other denitrifiers could utilize it directly as glucose. For these reasons, it is believed that glucose was being used by non-poly-P denitrifiers.

The acetate and unamended samples, however, showed no significant variation in specific denitrificatin rate with temperature (see figures 18 and 20). For this reason, it is believed that the bacteria denitrifying in these samples belonged to a different bacterial population than the denitrifiers in the glucose amended samples. In this research, the unamended samples were expected to exhibit the lowest denitrification rate, since they did not contain readily biodegradable COD as substrate for denitrification. Instead, it has been found that they may have used slowly biodegradable substrate obtained from the pilot plant influent. This conclusion is based upon the fact that the unamended denitrification rates were lower than those using RBCOD and higher than the rates obtained from the endogenous rate tests. Since it would take longer for the bacteria to break down SBCOD into usable forms, the overall rate of nitrate removal would be lower than if RBCOD were used as substrate.

At 10 degrees, the acetate and glucose amended samples showed similar denitrification rates, with glucose remaining slightly higher. It is believed that the similar rates were the result of a change in the bacterial population. As found in work by Sell et al. (1981), lower temperatures gave rise to a population shift within the phosphorus removing community. The wide variety of different poly-P bacteria include psychrophiles, which at lower temperatures become the predominant species of bacteria within the poly-P community. If these bacteria were also capable of denitrifying, then lower temperatures may enable the poly-P bacteria to denitrify at higher rates than the other bacteria, possibly due to the poly-P bacteria having an advantage over other denitrifying bacteria in obtaining COD. Other denitrifiers in the activated sludge community are believed to be predominantly mesophiles (Randall, 1996), which exhibit lower nitrate removal rates at lower temps, as found in previous work (Sutton (1975); Dawson and Murphy (1972); and Stensel (1973)).

In the 8 degree batch tests, the shift in glucose denitrification rates continued. At this temperature, the acetate amended samples had the highest nitrate removal rates, followed by glucose and the unamended assays. As shown in figure 18, denitrification

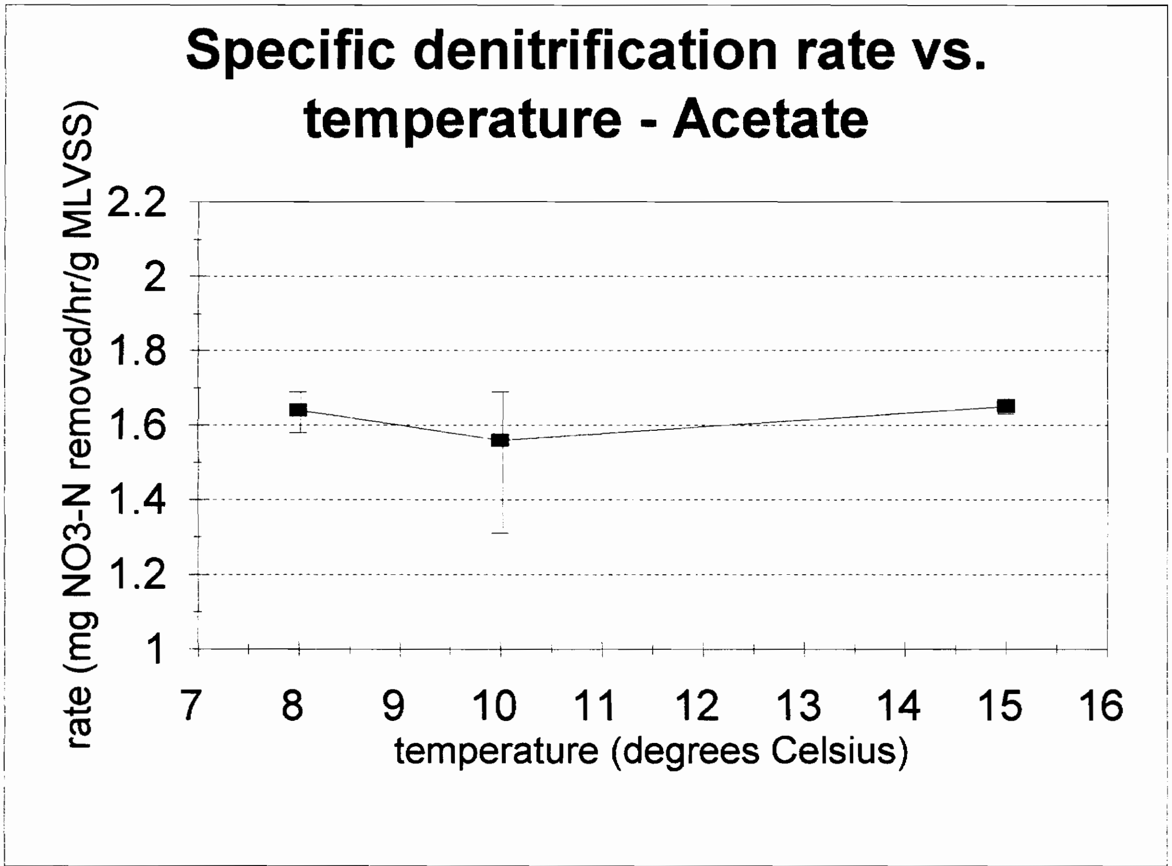

Figure 18. Specific denitrification rates as a function of temperature - Acetate.

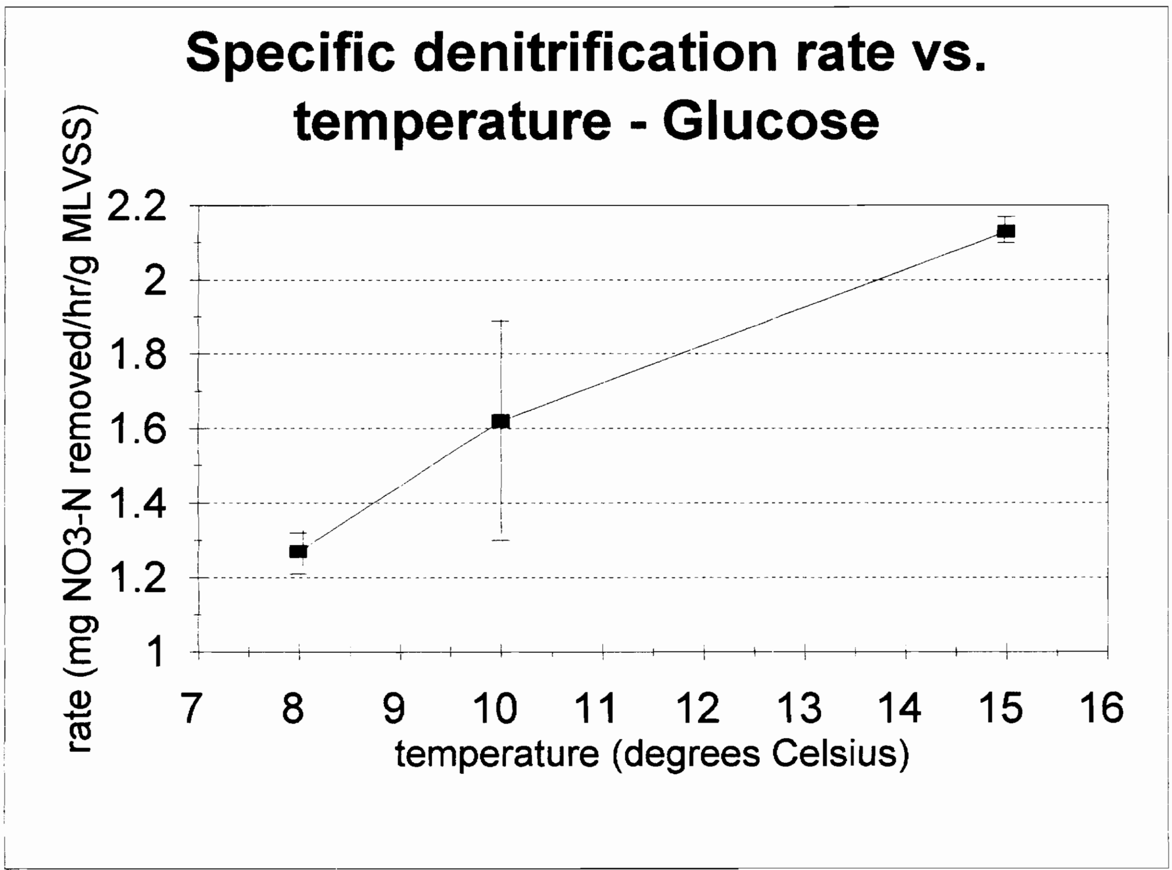

Figure 19. Specific denitrification rates as a function of temperature - Glucose.

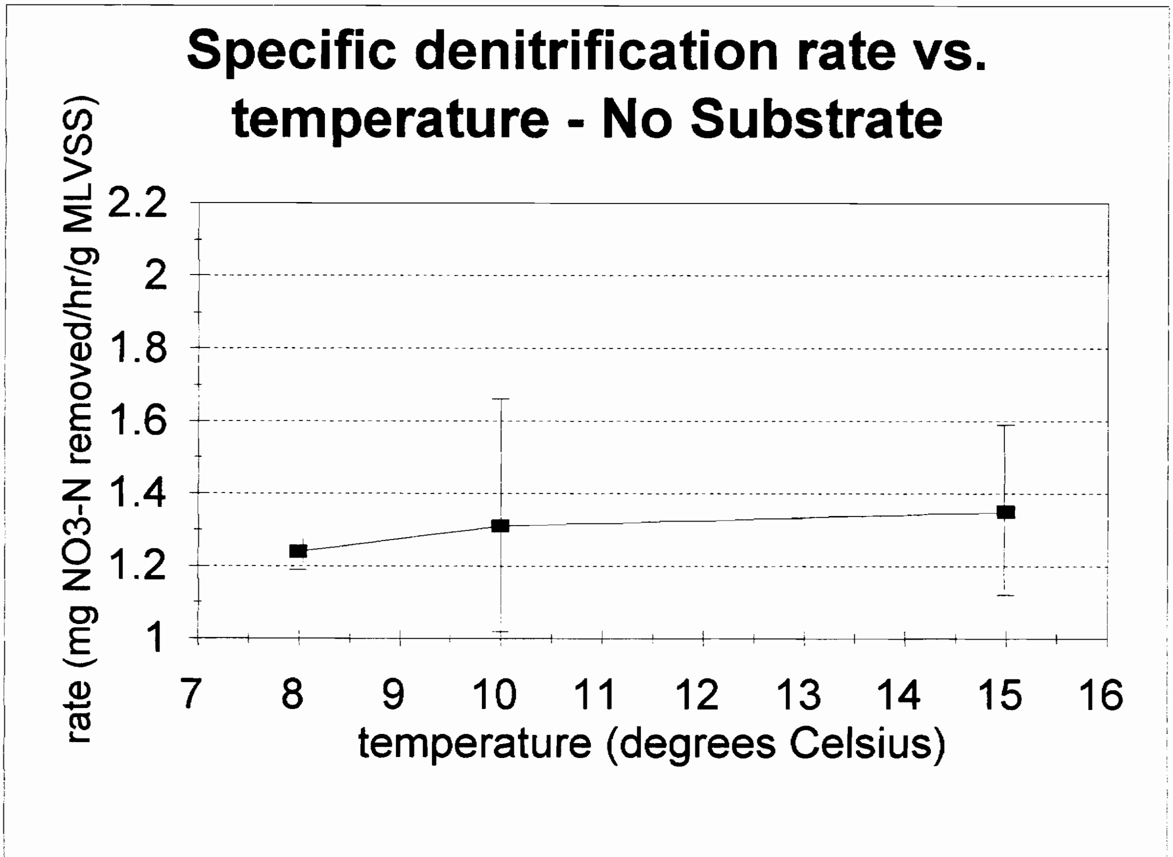

Figure 20. Specific denitrification rates as a function of temperature - No Substrate.

rates of the acetate amended samples remained constant throughout the temperatures investigated. It is believed that this is due to a population shift within the poly-P community. As temperature decreased, the poly-P bacterial composition shifted such that a larger mass of psychrophilic poly-P bacteria were present in solution.

The glucose amended samples, however, showed a decrease in nitrate removal rates as the temperature decreased (see figure 19). This is possibly due to the fact that non-poly-P denitrifiers are believed to be primarily mesophiles. Since, at 8 degrees, the glucose and unamended samples had similar rates, it is possible that the denitrifiers that were using glucose were inhibited by the lower temperatures such that their overall utilization of glucose, and therefore their denitrification rate, decreased dramatically.

Determination of carbon source in unamended samples

In the samples to which there was no substrate added, denitrification occurred at a constant rate throughout the experiment. The rates obtained from these samples were representative of bacteria using internal substrates, slowly biodegradable COD or decay products as carbon sources.

The PHA results from all of the batch tests were found to be inconclusive, regardless of substrate. There did not appear to be any significant reduction in PHAs as a result of the denitrification process. This finding was supported by Clayton et al. (1990), who also found no significant trend in PHA levels. Several reasons for the lack of a PHA trend can be postulated. First, the PHA method was not accurate enough to detect changes in PHA concentrations, if these changes were relatively small (< 1 % TSS). Therefore, some samples may actually have exhibited subtle trends that this method could not detect. Second, it is possible that PHA was not being used in the denitrification process. If this were true, it would mean that either poly-P bacteria cannot denitrify, as previously suggested (Comeau et al., 1987; Vlekke et al., 1988; and Kuba, 1993), or that they accomplished denitrification with another carbon source, possibly external. Third, it is possible that PHA production and utilization were occurring simultaneously, such that no noticeable trend appeared in the PHA concentration. Finally, it is possible that PHAs were being utilized as substrate, but that the percentage on the basis of total biomass remained constant. In this scenario, the poly-P bacteria would use PHAs as a carbon source while the non-poly-P denitrifiers would rely on endogenous decay products to provide carbon for substrate. Therefore, the PHA concentration and MLVSS would both decrease such that the ratio of PHA to MLVSS remained essentially constant throughout the experiments. These possible scenarios are addressed below.

An endogenous test was performed to determine the ultimate fate of PHAs during denitrification. If PHAs were utilized and produced simultaneously, then PHA concentrations should decrease in the absence of all substrate, since no PHA production can occur. Furthermore, if PHAs were used for denitrification purposes, a disappearance in PHAs would be expected to take place in a long term denitrification test, coupled with an uptake of phosphorus from solution. During endogenous test #1, there was no substrate remaining in solution, and yet PHA concentrations persisted while there was an electron acceptor available. After ten hours, the PHA data became rather variable, possibly due to the statistical variations in the PHA determination method. Since PHAs did not disappear completely during this test, it was believed that they were not being used as a denitrification substrate at 10 degrees. Since this was considered close to the optimum operating temperature for poly-P bacteria (von Consbruch, 1995), and PHAs were not utilized for denitrification at this temperature, it was reasonable to conclude that they were not used at all.

Another test was performed to track the changes of PHA and MLVSS concentrations over time. Endogenous test # 2 was also performed without the addition of substrate, to allow the samples to become endogenous. If the concentrations of MLVSS and PHAs decreased at similar rates, such that their ratio (PHA/MLVSS) remained constant throughout, then changes in PHA concentrations would not be noticed. In this case, non-poly-P bacteria may undergo endogenous respiration, reducing MLVSS concentrations, while poly-P bacteria are utilizing PHA reserves to denitrify. In this test, it was found that MLVSS, PHA, and phosphorus concentrations showed no significant changes during the time of the experiment. Furthermore, it was found that PHA concentrations did not show any significant trend after 10 hours. This would appear to support the results of endogenous rate test # 1, which found that the PHA concentrations did not disappear during the test. Also, the concentrations of PHAs that were detected during these tests were subject to high variability due to the experimental error associated with the PHA method.

It is hypothesized that poly-P bacteria may not be capable of utilizing all of the PHAs found within the cell. If this were the case, then many of the PHA values obtained in this research may be residual PHA which was unavailable to the cell for use as a denitrification substrate. The lower limit of PHA concentrations found in these tests was usually around 2 - 3 % TSS. PHA levels within this range may represent the effective absence of available PHA. Since many of the PHA concentrations fell within this range, it is possible that the poly-P bacteria were not denitrifying using internal substrates because there were none available within the cell.

Since it appears PHAs were not used for denitrification, then another carbon source must have been present. In order to determine the identity of this carbon source, further endogenous and SBCOD tests were performed. Endogenous rate test # 3 (figure 16) was performed to determine the rate at which bacteria denitrify endogenously. Since PHAs were not believed to be used as substrate for denitrification, it was believed that the nitrate removal rates obtained from unamended samples that were allowed to react for 28 hours before sampling were representative of endogenous denitrification rates. The rate obtained, 0.57 mg NO_3-N removed/ hr/g MLVSS, was found to be much lower than the unamended rates obtained previously, indicating that the rates obtained from the unamended batch tests were not representative of endogenous denitrification rates. The endogenous denitrification rate obtained was supported by Jones, et al. (1990), where the author stated that that endogenous denitrification rates rarely exceed 1 mg NO_3-N removed/g MLVSS/hr.

The rates obtained from the SBCOD rate test were just slightly lower than those obtained from the unamended batch tests (0.9 mg NO3-N removed/hr/g MLVSS for the SBCOD test versus 1.0-1.7 mg NO3-N removed/hr/g MLVSS for the unamended tests). Therefore, the unamended denitrification rates appear to be representative of a bacterial population that is both unaffected by temperature and capable of retaining SBCOD (either externally or internally, but not as PHA) and utilizing it as a carbon source for denitrification. This population may consist of the entire denitrifying population (including poly-Ps), or just a fraction thereof. Previous research by Lewandowski (1982) also resulted in the conclusion that the bacteria were utilizing SBCOD as substrate. In the study by Lewandowski (1982), unamended samples from an activated sludge plant were allowed to react for 2 hours before tests were begun. The results of these tests show that the denitrification rates of the unamended samples were nearly identical to those obtained using acetone as substrate, and very close to the rates obtained using methanol. The conclusion Lewandowski came to was that the substrate was SBCOD obtained from the activated sludge plant, which had no primary sedimentation.

The fact that acetate denitrification rates were higher than the unamended rates signifies that there is a bacterial population utilizing acetate as a denitrification substrate. If this population were the same as the bacterial population utilizing glucose, then a similar temperature dependency would be expected. The absence of this dependency suggests that these denitrifiers may be poly-P bacteria capable of denitrifying. Also, the natural affinity of poly-P bacteria to VFAs would probably enable them to obtain and consume VFAs more quickly and efficiently than other bacteria. This acetate is not being used by the same metabolic pathways involved in PHA production/consumption, since no significant change in PHA concentrations take place. It is therefore believed that the acetate is being utilized in a different manner. Lotter (1986) and Kunst (1991, according to Ante, 1994) have both concluded that some poly-P bacteria are capable of growth on substrates other than PHAs, provided an electron acceptor is available. Therefore, it is believed that a portion of the poly-P population are removing nitrate using acetate as substrate in a manner similar to the denitrification process of other non-poly-P denitrifiers.

In this research, the glucose amended samples yielded a temperature correction constant (Θ) of 1.02. This number was calculated using the equation

$$\Theta^{t2 - t1} = k_{t2} / k_{t1} \text{ , where}$$

k_{t1} = denitrification rate at temperature 1

k_{t2} = denitrification rate at temperature 2

This equation is used to characterize the effect of temperature on kinetic processes. The temperature correction constant was found to be lower than those obtained in previous work (see Table 10). The reason for this was perhaps three-fold. First, this discrepancy may be due to a lower concentration of denitrifiers in the mixed liquor. Since the mixed liquor used in these experiments was obtained from a system which removes both nitrogen and phosphorus, the total mass of denitrifiers in a unit volume of mixed liquor was lower. In other research (Sutton (1973); Dawson and Murphy (1972); Stensel (1970); and Lewandowski (1982)), the mass of denitrifiers in a unit volume of mixed liquor was probably significantly higher, since they were obtained from plants that do not remove phosphorus, only nitrogen. Therefore, normalizing the denitrification rates to MLVSS is not necessarily normalizing these rates to the mass of denitrifiers in solution. Second, denitrification rates will be affected by the substrate used. None of this previous research has been done using glucose as substrate, so any direct comparisons would be difficult at best. Finally, in the temperature range that was studied, a plot of the Arrhenius equation showed that the temperature dependency of denitrification was nearly linear, as these tests have concluded. If these tests were run over a broader temperature range, a higher temperature correction factor may have been obtained.

The Arrhenius activation energy (E_a) obtained from the glucose amended samples was 11,800 cal/mol. As shown in Table 10, this value correlated rather well with previous research. The specific denitrification rates obtained from this research varied according to temperature and substrate used. In general, these ranged from 1.2 to 2.1 mg NO_3-N removed/g MLVSS/hr. The reasons for discrepancies and similarities between these rates and those reported by others were the same as outlined above for the temperature correction constant.

PHA generation versus temperature

The PHA analysis performed during this research indicated that the ratio of PHV to PHB in a given batch test remained constant throughout the test, regardless of substrate used. While these ratios varied from 0.10 to 0.50, most fell within the 0.20 to 0.30 range. In all but one test, duplicate samples also had approximately the same ratios of PHV to PHB. In previous work it has been shown that the type of VFA used (acetate, propionate, etc.) may impact this ratio (Comeau, 1987), and it would appear from this work and the work of others that there is only minor variation in this ratio, possibly caused by the specific composition of the poly-P community. In work by Liu (1994), this ratio was 0.30 when acetate was used, while Comeau (1987) found that this ratio ranged from 0.16 to 0.36.

It was also found in this work that PHA generation rates varied according to temperature (see figure 21). As shown, the poly-P bacteria obtained appeared to contain lower initial concentrations of PHAs at lower temperatures (expressed as % TSS). It was believed that this may have been due to an increased efficiency at lower temperatures by poly-P bacteria within the pilot plant.

Table 10. Previous research into temperature effects on biological nutrient removal and specific denitrification rates - a summary.

Author(s)	Bacterial culture	Temp. (°Celsius)	Θ (temp. correction constant (dimension-less)	Arrhenius activation energy (E_a) (cal/mol)	Specific denit. rates (mg NO_3-N removed/hr/g MLVSS)	Substrate
Dawson and Murphy (1972)	dominant Pseudomonas denitrificans	5-27	1.12	16800	10 degrees: 28.5 15 degrees: 35.2	sodium citrate
Kuba (1993)	BPR activated sludge	25	N/A	N/A	18.4	methanol
Sutton (1973)	activated sludge	6-25	1.09	15900	10 degrees: 2.0 15 degrees: 4.0	wastewater w/methanol
Stensel (1970)	activated sludge	10-20	1.06	10000	unavailable	methanol
Mulbarger (1970)[1]	activated sludge	10-20	1.15	19000	unavailable	wastewater
Murphy and Sutton (1974)[1]	activated sludge	6-25	1.07	unavailable	unavailable	wastewater
Lewandowski (1982)	activated sludge	5-35	1.08 1.07	11000-12000	10 deg.: 0.6 - 2.2 20 deg.: 1.3 - 4.2	unamended methanol, acetic acid, or acetone
Jones (1990)	activated sludge	20	N/A	N/A	1.7 - 4.1	molasses waste, urea, peptone, milk, starch

Note: None of the bacterial cultures obtained from activated sludge plants were obtained from plants that remove phosphorus biologically.

[1]The data representing these articles was obtained from Christensen (1977)

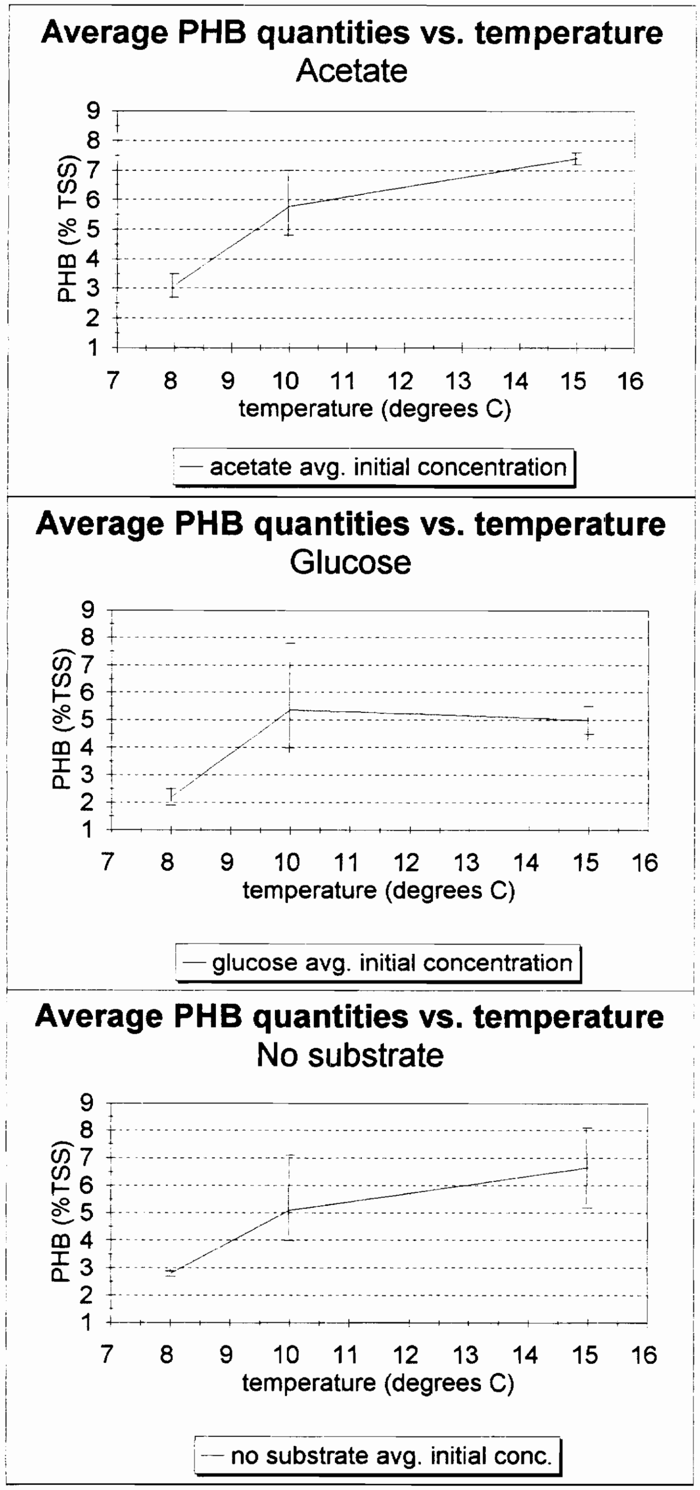

Figure 21. PHB generation as a function of temperature.

CHAPTER SIX: SUMMARY AND CONCLUSIONS

Summary

While the results of this work suggest that PHAs were not being used as substrates for denitrification, it does not mean that poly-P bacteria are incapable of denitrifying. Many of the bacteria that are capable of removing phosphorus may also be capable of denitrifying using some other substrate, possibly external. The results of this research imply that poly-P bacteria may be capable of denitrification when conditions are favorable.

Conclusions

- PHB showed no apparent trend over the three hour batch test, regardless of which substrate was added. Furthermore, PHB was not utilized as a denitrification substrate in an endogenous test. Therefore, PHB did not appear to be used as a denitrification substrate by the poly-P bacteria.

- As temperature decreased, denitrification rates using glucose as substrate decreased, indicating that glucose was probably being used by a bacterial population whose optimum denitrification temperature was mesophilic. This would imply that this population was not simply poly-P bacteria since previous work has shown that poly-P bacteria are nearly unaffected by changes in temperatures in the range studied in this research (8° C - 15° C). Therefore, the population capable of denitrifying under these conditions is believed to have been primarily non-poly-P bacteria. Furthermore, this shift in temperatures was similar to others found when non-poly-P denitrifiers were studied.

- As temperature decreased, denitrification rates using acetate showed no significant change. This lack of a trend implied the denitrifying bacteria may have been poly-P bacteria, since they have been shown to be only slightly affected by temperature changes in the range studied in this research. Furthermore, these poly-P bacteria probably had an advantage over other bacteria in obtaining and utilizing VFAs. Therefore, it is believed that the denitrifying bacterial population in the acetate amended samples may have been predominantly poly-P bacteria. These bacteria were using acetate without producing PHAs. Rather, they may have utilized it much as other denitrifiers utilize external substrates.

- Kinetic rates obtained from the unamended assays appear to be representative of denitrification rates obtained for bacteria using slowly biodegradable substrate (SBCOD). This slowly biodegradable substrate was obtained from the UCT system and remained available to the bacteria throughout the duration of the batch test. Endogenous denitrification rates were found to be much lower than the unamended denitrification rates.

- As temperature decreased, denitrification rates (per g MLVSS) obtained from the unamended assays decreased only slightly. This would seem to indicate that the denitrifying bacterial population may have been predominantly poly-P bacteria, since the effect of temperature changes did not significantly alter the denitrification rate of the mixed liquor.

- In each of these batch tests, one denitrification rate was observed throughout the test. In previous work (van Haandel, et. al.(1981); Clayton, et. al.(1991); and Barnard (1976)), two different denitrification rates have been observed, one for bacteria using both readily and slowly biodegradable substrates, and one for bacteria using slowly biodegradable substrate only. In the unamended tests performed in this study, there was only one observed denitrification rate since the readily biodegradable substrate was consumed prior to injection of nitrate.

- Poly-betahydroxyvalerate was analyzed using the PHA analysis method used by Hart. PHV/PHB ratios were essentially constant for each sample of every batch test run. These values, however, showed no apparent correlation between the different batch tests, regardless of temperature, date of batch test, etc. What factors influenced this ratio are unclear at this time.

- PHA production rates (starting PHA concentrations in batch tests) decreased with a decrease in temperature, though it has been shown that phosphorus removal rates are highest at temperatures around 10 degrees. This trend may have been due to an increased efficiency of poly-P bacteria at lower temperatures.

Engineering significance

The results of this research may be useful in the future design of biological nutrient removal systems. These results indicate that biological nutrient removal systems which remove both phosphorus and nitrogen may exhibit higher denitrification rates at lower temperatures than systems which remove only nitrogen. Therefore, biological nutrient removal systems operating in cold climates may remove nitrate more efficiently when removing both nitrogen and phosphorus.

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APPENDIX

Batch 7/4/95 - 15 degrees C
NITRATE

acetate

time	area	conc	regress
0.033333	1.77E+07	16.34646	15.48509
0.333	1.63E+07	15.05591	14.21152
0.667	1.27E+07	11.72336	12.79204
1	1E+07	9.293857	11.37681
1.333	1.12E+07	10.38016	9.961572
1.667	9636458	8.922461	8.542089
2	8395833	7.773758	7.126856

Regression Output:	**Acetate NO3-N**
Constant	15.62676
Std Err of Y Est	1.238879
R Squared	0.877583
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-4.24995
Std Err of Coef.	0.709864

glucose

time	area	conc	regress
0.03333	1.73E+07	16.02656	16.02643
0.333	1.54E+07	14.27184	14.38685
0.667	1.33E+07	12.32412	12.55945
1	1.13E+07	10.49325	10.73751
1.333	1.14E+07	10.56668	8.915581
1.667	6514211	6.031552	7.088176
2	6314607	5.846737	5.266243

Regression Output:	**Glucose NO3-N**
Constant	16.20878
Std Err of Y Est	0.996353
R Squared	0.934426
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	-5.47127
Std Err of Coef.	0.724689

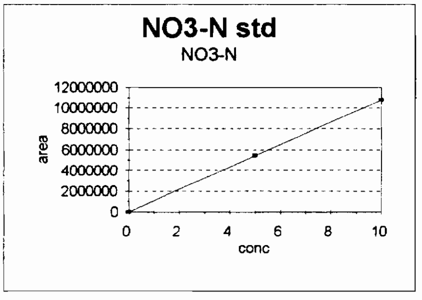
No substrate

time	area	conc	regress
0.03333	1.76E+07	16.33526	15.22704
0.333	1.61E+07	14.94175	14.35746
0.667	1.45E+07	13.40643	13.38826
1	9336323	8.644564	12.42196
1.333	1.29E+07	11.94131	11.45566
1.667	1.22E+07	11.31446	10.48646
2	1.11E+07	10.27324	9.520167

Regression Output:	**No substrate NO3-N**
Constant	15.32375
Std Err of Y Est	1.861566
R Squared	**0.596803**
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-2.90179
Std Err of Coef.	1.066656

Nitrate standard

conc	area	regress
0	0	0
5	5432058	5400112
10	1.08E+07	1.08E+07



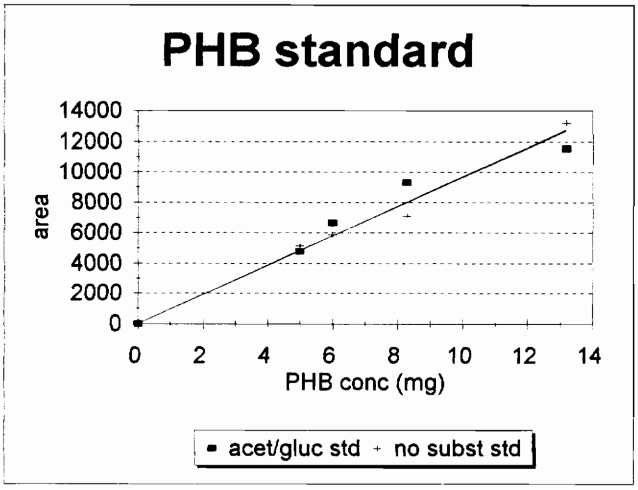
Regression Output:	**Nitrate standard**
Constant	0
Std Err of Y Est	25255.53
R Squared	0.999978
No. of Observations	3
Degrees of Freedom	2
X Coefficient(s)	1080022
Std Err of Coef.	2258.923

PHB std

vial	conc	area 1	regress 1	area2	regress 2
13	0	0	0	0	0
12	5	4770.09	4821.759	5133.13	4840.105
9	6	6634.17	5786.111	5863.92	5808.126
8	8.3	9292.21	8004.12	7107.59	8034.575
11	13.2	11553.6	12729.44	13224.4	12777.88

Two regressions are shown because PHB samples were run at 2 different times due t(a limited number of vials.

Regression Output:	**PHB regression 1**	Regression Output:	**PHB regression 2**
Constant	0	Constant	0
Std Err of Y Est	970.0076	Std Err of Y Est	535.6443
R Squared	0.952106	R Squared	0.987225
No. of Observations	5	No. of Observations	5
Degrees of Freedom	4	Degrees of Freedom	4
X Coefficient(s)	964.3519	X Coefficient(s)	968.0211
Std Err of Coef.	55.62186	Std Err of Coef.	30.71474



the data points for the 2 regress lines are nearly identical

PHB Samples
acetate

time	area	conc	mass	%TSS	regress1	regress2	overall reg
0.03333	3296.02	3.41786	43	7.948512	8.196017		8.196017
0.333	2944.44	3.053284	34.5	8.850098	8.681438		8.681438
0.667	3389.45	3.514744	36.6	9.603126	9.222469		9.222469
1	3284.22	3.405624	36	9.460067	9.76188	9.360848	9.561364
1.333	2686.8	2.78612	36.7	7.591608		7.553542	7.553542
1.667	2147.75	2.227144	41.5	5.366611		5.740809	5.740809
2	1608.7	1.668167	40	4.170418		3.933503	3.933503

Regress1 is the regression of the first half of the data points (when the slope is positive
Regress2 is the regression of the second half of the data points(when the slope is nega
These data points appear to show linear trends, but in reality (as is shown in later tests
there may be no relationship at all.

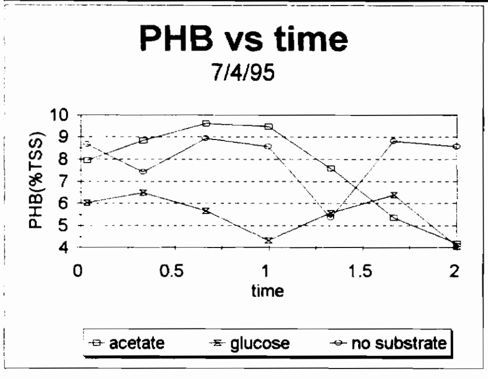
Regression Output:	**Regress 1**		Regression Output:	**Regress 2**	
Constant		8.142027	Constant		14.78819
Std Err of Y Est		0.403544	Std Err of Y Est		0.322061
R Squared		0.808273	R Squared		0.987487
No. of Observations		4	No. of Observations		4
Degrees of Freedom		2	Degrees of Freedom		2
X Coefficient(s)	1.619853		X Coefficient(s)	-5.42735	
Std Err of Coef.	0.557857		Std Err of Coef.	0.432003	

glucose

time	area	conc	mass	%TSS
0.03333	2252.83	2.336108	38.8	6.020897
0.333	2274.21	2.358278	36.3	6.496634
0.667	2049.2	2.124951	37.5	5.666535
1	1500.91	1.556392	36	4.323312
1.333	1827.22	1.894765	34	5.572838
1.667	2128.27	2.206943	34.5	6.396937
2	1466.54	1.520752	37.5	4.055339

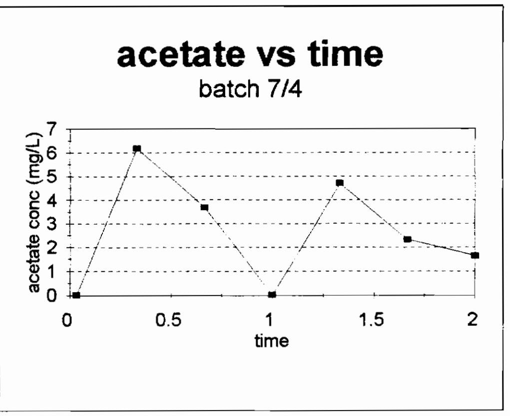
no substrate

time	area	conc	mass	%TSS
0.03333	3235.42	3.342303	38.5	8.681307
0.333	2614.45	2.711095	36.5	7.427659
0.667	3318.17	3.440829	38.5	8.937219
1	2856.81	2.962415	34.5	8.586709
1.333	1680.13	1.742238	32.5	5.360731
1.667	3058.27	3.171322	36	8.809227
2	3309.81	3.43216	40	8.5804



ACETATE

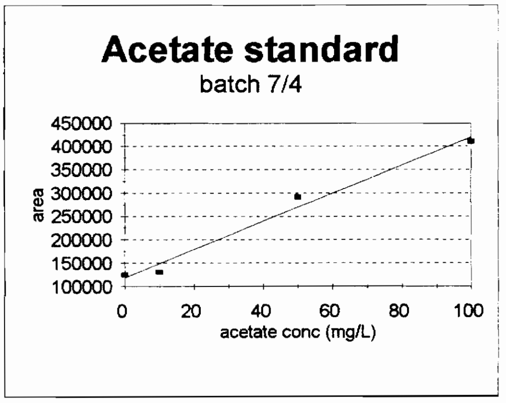
time	area	conc	conc(adj)
0.03333	116071	-1.01003	0
0.333	137716	6.18477	6.18477
0.667	130187	3.68213	3.68213
1	114892	-1.40193	0
1.333	133211	4.687308	4.687308
1.667	126121	2.330591	2.330591
2	124070	1.648839	1.648839



acetate std

conc	area	regress
0	124775	119109.6
10	130606	149193.8
50	291658	269530.7
100	410747	419951.9

Regression Output:	acetate std	
Constant		119109.6
Std Err of Y Est		21816.85
R Squared		0.983318
No. of Observations		4
Degrees of Freedom		2
X Coefficient(s)	3008.423	
Std Err of Coef.	277.0742	



Note: since the data point at 1 hour for nitrate in the no substrate assay was obviously
($r^2 = 0.59$) a regression was performed without that point, so as to improve the acc
of the line.

regress no substrate nitrate line without bad data point:

time	conc	regress
0.03333	16.33526	15.8633
0.333	14.94175	14.99166
0.667	13.40643	14.02016
1.333	11.94131	12.08297
1.667	11.31446	11.11147
2	10.27324	10.14288

Regression Output:		**NO3-N no substrate**
Constant		15.96025
Std Err of Y Est		0.412357
R Squared		0.974284
No. of Observations		6
Degrees of Freedom		4
X Coefficient(s)	-2.90868	
Std Err of Coef.	0.236277	

time	regressed conc
0.03333	15.8633
0.333	14.99166
0.667	14.02016
1	13.05156
1.333	12.08297
1.667	11.11147
2	10.14288

PHOSPHATE

acetate

time	area	conc	conc/10
0.03333	4.2E+07	102.5918	10.25918
0.333	4.2E+07	104.0005	10.40005
0.667	5.8E+07	142.7139	14.27139
1	6.2E+07	151.5745	15.15745
1.333	5.9E+07	145.5946	14.55946
1.667	5.6E+07	137.8189	13.78189
2	6E+07	147.4494	14.74494

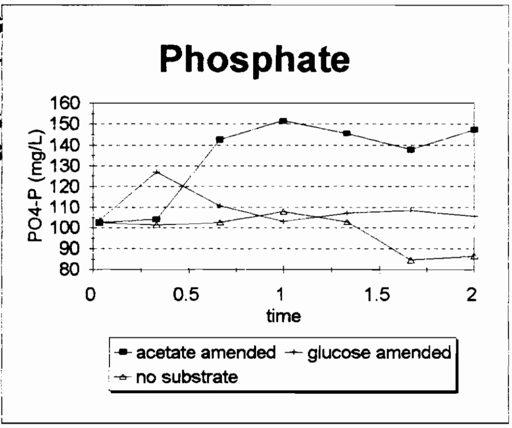
407909.1

glucose

time	area	conc	conc/10
0.03333	4.2E+07	103.838	10.3838
0.333	5.2E+07	126.9573	12.69573
0.667	4.5E+07	110.9025	11.09025
1	4.2E+07	103.0977	10.30977
1.333	4.4E+07	107.1589	10.71589
1.667	4.4E+07	108.541	10.8541
2	4.3E+07	105.6304	10.56304

no substrate

time	area	conc	conc/10
0.03333	4.2E+07	102.5458	10.25458
0.333	4.1E+07	101.4014	10.14014
0.667	4.2E+07	102.6576	10.26576
1	4.4E+07	108.0084	10.80084
1.333	4.2E+07	102.9251	10.29251
1.667	3.5E+07	84.63736	8.463736
2	3.5E+07	86.5898	8.65898



Batch 7/7/95 - 15 degrees C

NITRATE

acetate

time	area	conc	regress	Regression Output:	**acetate NO3-N**	
0	1.2E+07	10.89495	10.40665	Constant		10.40665
0.333	1.0E+07	9.367227	8.966134	Std Err of Y Est		0.621705
0.667	7771454	6.943266	7.521297	R Squared		0.979412
1	6581293	5.879938	6.080785	No. of Observations		7
1.5	3362806	3.004438	3.917854	Degrees of Freedom		5
2	2376764	2.123477	1.754923			
2.5	29470	0.026329	0	X Coefficient(s)	-4.32586	
3	19641	0.017548	0	Std Err of Coef.	0.280488	

glucose

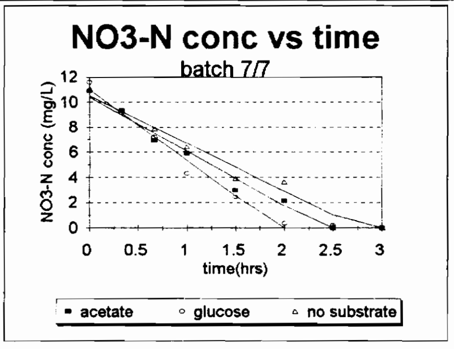
time	area	conc	regress	Regression Output:	**NO3-N glucose**	
0	1.3E+07	11.57629	10.99612	Constant		10.99612
0.333	1.0E+07	9.12627	9.12189	Std Err of Y Est		0.677335
0.667	8051576	7.193536	7.242031	R Squared		0.979472
1	4838041	4.322461	5.3678	No. of Observations		6
1.5	2729160	2.438319	2.55364	Degrees of Freedom		4
2	407511	0.364083	0			
2.5	221454	0.197854	0	X Coefficient(s)	-5.62832	
3	21735	0.019419	0	Std Err of Coef.	0.407405	

no substrate

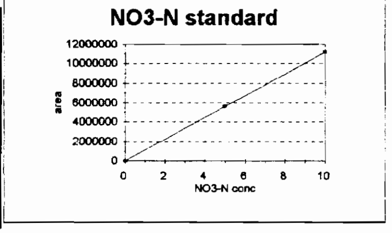
time	area	conc	regress	Regression Output:	**NO3-N no substrate**	
0	1.2E+07	11.08618	10.50552	Constant		10.50552
0.333	1.0E+07	9.333075	9.242731	Std Err of Y Est		0.763493
0.667	8782914	7.846936	7.976151	R Squared		0.970245
1	7166400	6.402691	6.713362	No. of Observations		8
1.5	4428363	3.956441	4.817283	Degrees of Freedom		6
2	4081161	3.64624	2.921204			
2.5	41783	0.03733	1.025125	X Coefficient(s)	-3.79216	
3	24099	0.021531	0	Std Err of Coef.	0.271111	

NO3-N standard

conc	area	regress
0	0	0
5	5613756	5596397
10	11184114	1.12E+07



Regression Output:	**NO3-N standard**	
Constant		0
Std Err of Y Est		13723.65
R Squared		0.999994
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	1119279	
Std Err of Coef.	1227.481	



PHB

acetate

time	mass	area	area/mass	%TSS
0.03333	50	1854.65	37.093	3.824371
0.333	53.5	2718.34	50.81009	5.238634
0.667	51.5	6500.71	126.2274	11.21633
1	51	5045.76	98.93647	8.79131
1.5	51	5085.71	99.7198	8.860915
2	49	4181.75	85.34184	7.583316
2.5	51	1989.21	39.00412	3.465833
3	51	4911.59	96.30569	8.557543

glucose

time	mass	area	area/mass	%TSS
0.03333	53	2029.69	38.29604	3.948407
0.333	50	1461.67	29.2334	3.014029
0.667	51.5	4122.4	80.0466	7.112791
1	49	3668.05	74.85816	6.651756
1.5	51	3039.66	59.60118	5.296049
2	51.5	2411.27	46.82078	4.160407
2.5	50	1862.98	37.2596	3.310818
3	52	1451.32	27.91	2.48003

no substrate

time	mass	area	area/mass	%TSS
0.03333	52	3355.83	64.53519	6.653723
0.333	52.5	2786.86	53.08305	5.472981
0.667	51	4268.09	83.68804	7.436363
1	50	3293.76	65.8752	5.853547
1.5	52	2883.5	55.45192	4.927354
2	50	2453.97	49.0794	4.361104
2.5	50	2176.35	43.527	3.867728
3	51.5	1724.38	33.48311	2.975246

PHV/PHB

acetate

time	PHB	PHV	PHV/PHB
0.03333	1854.65	386.16	0.208212
0.333	2718.34	582.78	0.214388
0.667	6500.71		0
1	5045.76		0
1.5	5085.71		0
2	4181.75	1059.66	0.253401
2.5	1989.21	455.89	0.229181
3	4911.59	1425.51	0.290234

glucose

time	PHB	PHV	PHV/PHB
0.03333	2029.69	574.7	0.283147
0.333	1461.67	356.37	0.24381
0.667	4122.4		0
1	3668.05		0
1.5	3039.66		0
2	2411.27	602.31	0.24979
2.5	1862.98	464.33	0.24924
3	1451.32	357.62	0.24641

no substrate

time	PHB	PHV	PHV/PHB
0.03333	3355.83	943.16	0.281051
0.333	2786.86	722.29	0.259177
0.667	4268.09		0
1	3293.76		0
1.5	2883.5		0
2	2453.97	623.39	0.254033
2.5	2176.35	519.19	0.23856
3	1724.38	386.53	0.224156

PHB std #1

conc	area	regress
0	0	0
5	5065.59	5626.947
6	5800.67	6752.337
8.3	6706.38	9340.732
13.2	17156.8	14855.14

Regression Output:	PHB standard 1	
Constant		0
Std Err of Y Est		1834.274
R Squared		0.914503
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	1125.389	
Std Err of Coef.	105.1803	

PHB std #2

conc	area	regress
0	0	0
5	5136.35	4849.555
6	5980.91	5819.466
8.3	7222.81	8050.262
13.2	13141.1	12802.83

Regression Output:	PHB standard 2	
Constant		0
Std Err of Y Est		476.2932
R Squared		0.989781
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	969.9111	
Std Err of Coef.	27.31145	

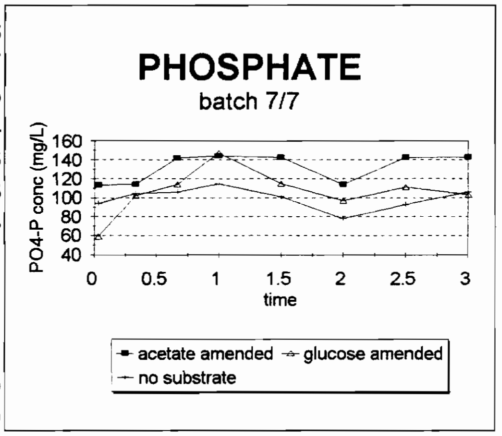
the second regression is for the first 2 samples of each type(@ t=0.033 and 0.33) because these samples were run separately from the rest.

PHOSPHATE

acetate

50 mg/L PO4-P std: 407909.1

time	area	conc	conc/10
0.03333	46147182	113.131	11.3131
0.333	46417951	113.7948	11.37948
0.667	57843829	141.8057	14.18057
1	58887615	144.3645	14.43645
1.5	58055440	142.3244	14.23244
2	46387248	113.7196	11.37196
2.5	58080279	142.3853	14.23853
3	58332052	143.0026	14.30026



glucose

time	area	conc	conc/10
0.03333	24041850	58.93923	5.893923
0.333	41696271	102.2195	10.22195
0.667	46562543	114.1493	11.41493
1	60224053	147.6409	14.76409
1.5	46589091	114.2144	11.42144
2	39529090	96.90661	9.690661
2.5	45324196	111.1135	11.11135
3	42090526	103.186	10.3186

no substrate

time	area	conc	conc/10
0.03333	38560578	94.53228	9.453228
0.333	42600604	104.4365	10.44365
0.667	43259469	106.0517	10.60517
1	46758217	114.629	11.4629
1.5	41089207	100.7313	10.07313
2	31878846	78.15183	7.815183
2.5	38007123	93.17547	9.317547
3	43318810	106.1972	10.61972

Batch 8/8 - 10 degrees C

NO3-N

acetate

time	area	conc	regress
0.08333	***1.1E+07***	10.45782	9.895994
0.333	7938405	7.54713	8.911165
0.667	9245584	8.78988	7.593694
1	6150634	5.847476	6.280167
1.5	5247508	4.988865	4.307905
2	1138926	1.08279	2.335642
2.5	1024478	0.973983	0.36338
3	96713	0.091946	0

sample at time 0.08333 was not filtered properly.
therefore, a starting concentration was assumed

Regression Output:	**acetate NO3-N**	
Constant		10.22469
Std Err of Y Est		1.113598
R Squared		0.922271
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-3.94452	
Std Err of Coef.	0.512119	
	1051844	

glucose

time	area	conc	regress
0.08333	1.2E+07	11.4281	10.70966
0.333	1.1E+07	10.32174	9.738951
0.667	8837745	8.402143	8.44037
1	***6418857***	6.102479	7.145676
1.5	3999969	3.802815	5.201692
2	4233124	4.024478	3.257708
2.5	1081792	1.028472	1.313724
3	70783	0.067294	0

Regression Output:	**glucose NO3-N**	
Constant		11.03364
Std Err of Y Est		0.918174
R Squared		0.958392
No. of Observations		8
Degrees of Freedom		6
X Coefficient(s)	-3.88797	
Std Err of Coef.	0.330722	

no substrate

time	area	conc	regress
0.08333	1.3E+07	12.98106	11.59413
0.333	1.1E+07	10.90966	10.82874
0.667	1E+07	9.778034	9.804818
1	8005825	7.757642	8.783964
1.5	7630384	7.39384	7.25115
2	3657598	3.544211	5.718336
2.5	3935945	3.813929	4.185522
3	4789471	4.640996	2.652708

Regression Output:	**no substrate NO3-N**	
Constant		11.84959
Std Err of Y Est		1.403727
R Squared		0.859688
No. of Observations		8
Degrees of Freedom		6
X Coefficient(s)	-3.06563	
Std Err of Coef.	0.505616	

NO3-N standard

conc	area	regress
0	0	0
5	4731956	5159960
10	1.1E+07	1E+07

Regression Output:	**NO3-N standard**	
Constant		0
Std Err of Y Est		338367.2
R Squared		0.995887
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	1031992	
Std Err of Coef.	30264.48	

PHB

acetate

time	mass	area	%TSS
0.08333	43	3801.42	5.500159
0.333	41.5	4318.25	6.473774
0.667	50	3130.75	3.895617
1	37.8	4070.38	6.699479
1.5	47.5	2790.56	3.655069
2	42.7	4405.04	6.418297
2.5	45.7	4083.23	5.558857
3	36	2928.69	5.061378

glucose

time	mass	area	%TSS
0.08333	45.5	6046.13	8.267304
0.333	44.5	5704.55	7.975524
0.667	41.5	4916	7.3699
1	49	4530.42	5.752283
1.5	39.5	4885.95	7.695729
2	49.5	3672.49	4.615867
2.5	42.3	4922.13	7.239533
3	47	4781.5	6.329423

no substrate

time	mass	area	%TSS
0.08333	44	4045.9	5.720847
0.333	43.5	3847.69	5.503116
0.667	48.2	3617.84	4.66982
1	34.7	3438.02	6.164201
1.5	51.5	3434	4.148498
2	31.7	3659.24	7.181737
2.5	41	3067.73	4.655123
3	40.5	4469.51	6.865983

PHV

acetate

time	mass	area	%TSS	PHV/PHB
0.08333	44	1315.92	1.860693	0.338298
0.333	43.5	1568.48	2.243301	0.346521
0.667	48.2	1020.89	1.31774	0.338262
1	34.7	1386.92	2.486679	0.371175
1.5	51.5	1073.31	1.296629	0.354748
2	31.7	1450.79	2.847365	0.443632
2.5	41	1402.01	2.127478	0.382719
3	40.5	950.9	1.460756	0.288608
			avg =	0.357996

glucose

time	mass	area	%TSS	
0.08333	44	1811.33	2.561196	0.309798
0.333	43.5	1939.28	2.773634	0.347768
0.667	48.2	1520.7	1.962883	0.266338
1	34.7	1457.12	2.612544	0.454175
1.5	51.5	1665.25	2.011732	0.261409
2	31.7	1665.54	3.26884	0.708175
2.5	41	1928.07	2.925747	0.404135
3	40.5	1851.08	2.843597	0.449266
			avg =	0.400133

no substrate

time	mass	area	%TSS	
0.08333	44	1468.2	2.076015	0.362886
0.333	43.5	1547.74	2.213638	0.402252
0.667	48.2	1426.73	1.841589	0.39436
1	34.7	1162.3	2.083947	0.338072
1.5	51.5	1292.59	1.561534	0.376409
2	31.7	1116.06	2.190414	0.304998
2.5	41	1165.46	1.768526	0.37991
3	40.5	1573.92	2.417828	0.352146
			avg =	0.363879

PHB standard

mass	PHB mass	area	regress
0	0	0	0
5	3.8	5871.27	6107.813
6.5	4.94	8185.51	7940.157
13.5	10.26	14790.4	16491.1
14	10.64	18712.4	17101.88

Regression Output:	**PHB standard**	
Constant		0
Std Err of Y Est		1183.459
R Squared		0.974302
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	1607.319	
Std Err of Coef.	73.77611	

PHV standard

mass	PHV mass	area	regress
0	0	0	0
5	1.2	4159.49	3855.403
6.5	1.56	4974	5012.024
13.5	3.24	10430.1	10409.59
14	3.36	10684.4	10795.13

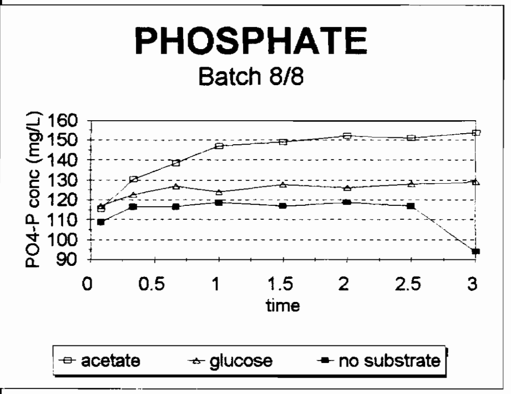
Regression Output:	**PHV standard**	
Constant		0
Std Err of Y Est		163.2454
R Squared		0.9987
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	3212.836	
Std Err of Coef.	32.22596	

PHOSPHATE

acetate

time	area	conc	conc/10
0.08333	4.35E+07	115.5009	11.55009
0.333	4.92E+07	130.5514	13.05514
0.667	5.22E+07	138.5638	13.85638
1	5.54E+07	147.0772	14.70772
1.5	5.62E+07	149.121	14.9121
2	5.74E+07	152.3621	15.23621
2.5	5.7E+07	151.2586	15.12586
3	5.8E+07	153.9873	15.39873

at time 0.0833 hr, sample was not filtered properly.



glucose

time	area	conc	
0.08333	4.4E+07	116.8656	11.68656
0.333	4.62E+07	122.6829	12.26829
0.667	4.79E+07	127.029	12.7029
1	4.68E+07	124.2212	12.42212
1.5	4.82E+07	127.9647	12.79647
2	4.76E+07	126.2669	12.62669
2.5	4.83E+07	128.2486	12.82486
3	4.87E+07	129.2415	12.92415

no substrate

time	area	conc	conc/10
0.08333	4.34E+07	108.7376	10.87376
0.333	4.65E+07	116.5211	11.65211
0.667	4.65E+07	116.5009	11.65009
1	4.74E+07	118.6694	11.86694
1.5	4.66E+07	116.8836	11.68836
2	4.74E+07	118.7551	11.87551
2.5	4.66E+07	116.7474	11.67474
3	3.76E+07	94.16305	9.416305

Phosphate standard 1

conc	area	regress
0	0	0
10	3192408	3768624
50	1.9E+07	1.88E+07

Regression Output:	**PO4-P standard 1**	
Constant		0
Std Err of Y Est		415515.5
R Squared		0.998324
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	376862.4	
Std Err of Coef.	8148.93	

Phosphate standard 2

conc	area	regress
0	0	0
10	3347541	3768624
50	2.01E+07	1.88E+07

Regression Output:	**PO4-P standard 2**	
Constant		0
Std Err of Y Est		463476.3
R Squared		0.998144
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	399026.7	
Std Err of Coef.	9089.518	

PHB duplicate

acetate-PHB duplicate

time	mass	area	%TSS
0.08333	32.5	3780.42	10.82782
0.333	44.3	5131.18	10.78196
0.667	47.5	4508.25	8.834837
1	37	3106.08	7.81439
1.5	41	3988.49	9.055425
2	46.5	4652.58	9.313761
2.5	46.7	4117.99	8.208288
3	40	2347.98	5.464099

glucose-PHB duplicate

time	mass	area	
0.08333	45	3995.83	9.22518
0.333	37	3740.74	9.411091
0.667	38.3	4028.31	9.790577
1	47	averaged	9.020866
1.5	48.8	4325.64	8.251155
2	40	3518.16	9.137681
2.5	39.3	3691.02	9.757403
3	46.2	2617.5	5.886066

no substrate-PHB duplicate

time	mass	area	%TSS
0.08333	41.7	3380.43	8.42202
0.333	39	4533.46	12.07662
0.667	49.5	4621.45	9.699591
1	41.6	3120.48	7.793068
1.5	44.5	3163.91	7.386598
2	38.2	3251.64	8.843404
2.5	49	4098.95	8.690743
3	43.7	2484.72	5.907128

PHB standard

conc	area	regress
0	0	0
2.736	2855.57	2633.514
3.648	4058.93	3511.353
5.168	5478.16	4974.416
10.412	9521.75	10021.99

PHV standard

conc	area	regress
0	0	0
0.864	1125.09	1047.093
1.152	1596.39	1396.124
1.632	2204.76	1977.843
3.288	3781.48	3984.772

PHV duplicate

acetate-PHV duplicate

time	mass	area		PHV/PHB
0.08333	32.5	1003.8	2.548544	0.23537
0.333	44.3	1403.09	2.613426	0.242389
0.667	47.5	1354.32	2.352643	0.266292
1	37	821.82	1.83275	0.234535
1.5	41	1160.26	2.335069	0.257864
2	46.5	1515.15	2.68863	0.288673
2.5	46.7	1183.51	2.091141	0.25476
3	40	651	1.342918	0.245771
			avg =	0.253207

glucose-PHV duplicate

time	mass	area	%TSS	PHV/PHB
0.08333	45	1164.13	2.134604	0.231389
0.333	37	1171.79	2.613223	0.277675
0.667	38.3	1338.64	2.883987	0.294568
1	47	averaged	2.378664	0.263685
1.5	48.8	1107.92	1.873341	0.22704
2	40	1008.73	2.080862	0.227723
2.5	39.3	1220.62	2.562809	0.262653
3	46.2	756.95	1.351928	0.229683
			avg =	0.251802

no substrate-PHV duplicate

time	mass	area	%TSS	PHV/PHB
0.08333	41.7	951.25	1.882292	0.223496
0.333	39	1592.01	3.36829	0.27891
0.667	49.5	1256.62	2.094725	0.21596
1	41.6	802.49	1.591749	0.204252
1.5	44.5	871.57	1.616109	0.218789
2	38.2	858.74	1.854926	0.209753
2.5	49	1255.32	2.113911	0.243237
3	43.7	686.25	1.295774	0.219358
			avg =	0.226719

Regression Output:	PHB standard	
Constant		0
Std Err of Y Est		461.8287
R Squared		0.982679
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	962.5418	
Std Err of Coef.	36.98638	

Regression Output:	PHV standard	
Constant		0
Std Err of Y Est		186.4193
R Squared		0.982201
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	1211.914	
Std Err of Coef.	47.27746	

average PHB

acetate

time	%TSS 1	%TSS 2	mass 1	mass 2	average
0.08333	5.50	10.83	43	32.5	7.79
0.333	6.47	10.78	41.5	44.3	8.70
0.667	3.90	8.83	50	47.5	6.30
1	6.70	7.81	37.8	37	7.25
1.5	3.66	9.06	47.5	41	6.16
2	6.42	9.31	42.7	46.5	7.93
2.5	5.56	8.21	45.7	46.7	6.90
3	5.06	5.46	36	40	5.27

glucose

time	%TSS 1	%TSS 2	mass 1	mass 2	average
0.08333	8.27	9.23	45.5	45	8.74
0.333	7.98	9.41	44.5	37	8.63
0.667	7.37	9.79	41.5	38.3	8.53
1	5.75	9.02	49	47	7.35
1.5	7.70	8.25	39.5	48.8	8.00
2	4.62	9.14	49.5	40	6.64
2.5	7.24	9.76	42.3	39.3	8.45
3	6.33	5.89	47	46.2	6.11

no substrate

time	%TSS 1	%TSS 2	mass 1	mass 2	average
0.08333	5.72	8.42	44	41.7	7.04
0.333	5.50	12.08	43.5	39	8.61
0.667	4.67	9.70	48.2	49.5	7.22
1	6.16	7.79	34.7	41.6	7.05
1.5	4.15	7.39	51.5	44.5	5.65
2	7.18	8.84	31.7	38.2	8.09
2.5	4.66	8.69	41	49	6.85
3	6.87	5.91	40.5	43.7	6.37

average PHV

acetate

time	%TSS 1	%TSS 2	mass 1	mass 2	average
0.08333	1.86	0.96	44	32.5	1.48
0.333	2.24	0.99	43.5	44.3	1.61
0.667	1.32	0.89	48.2	47.5	1.10
1	2.49	0.69	34.7	37	1.56
1.5	1.30	0.88	51.5	41	1.11
2	2.85	1.01	31.7	46.5	1.76
2.5	2.13	0.79	41	46.7	1.41
3	1.46	0.51	40.5	40	0.99

glucose

time	%TSS 1	%TSS 2	mass 1	mass 2	average
0.08333	2.56	0.81	44	45	1.67
0.333	2.77	0.99	43.5	37	1.95
0.667	1.96	1.09	48.2	38.3	1.58
1	2.61	0.90	34.7	47	1.63
1.5	2.01	0.71	51.5	48.8	1.38
2	3.27	0.78	31.7	40	1.88
2.5	2.93	0.97	41	39.3	1.97
3	2.84	0.51	40.5	46.2	1.60

no substrate

time	%TSS 1	%TSS 2	mass 1	mass 2	average
0.08333	2.08	1.42	44	41.7	1.76
0.333	2.21	2.54	43.5	39	2.37
0.667	1.84	1.58	48.2	49.5	1.71
1	2.08	1.20	34.7	41.6	1.60
1.5	1.56	1.22	51.5	44.5	1.40
2	2.19	1.40	31.7	38.2	1.76
2.5	1.77	1.59	41	49	1.67
3	2.42	0.98	40.5	43.7	1.67

Batch 8/9 - 10 degrees

NO3-N

acetate

time	area	conc	regress
0.083333	12773968	12.28241	11.49438
0.333	11025398	10.60113	10.23057
0.667	8059782	7.749632	8.539863
1	6817765	6.555409	6.854222
1.5	3721969	3.578743	4.323229
2	1726220	1.659793	1.792235
2.5	71366	0.06862	0
3	13268	0.012757	0

Regression Output:	**acetate**	
Constant		11.91621
Std Err of Y Est		0.734265
R Squared		0.978235
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-5.06199	
Std Err of Coef.	0.337673	

glucose

time	area	conc	regress
0.083333	12565141	12.08162	10.88669
0.333	11274437	10.84058	9.683675
0.667	7930059	7.624901	8.074298
1	7622425	7.329105	6.46974
1.5	4674572	4.494689	4.060493
2	780810	0.750763	1.651245
2.5	794446	0.763875	0
3	67543	0.064944	0

Regression Output:	**glucose**	
Constant		11.28823
Std Err of Y Est		0.492157
R Squared		0.989089
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-4.81849	
Std Err of Coef.	0.226332	

no substrate

time	area	conc	regress
0.083333	12910053	12.27373	12.5649
0.333	11457803	10.89306	11.32021
0.667	10413689	9.900411	9.65509
1	8724481	8.294462	7.994953
1.5	6684084	6.354633	5.502255
2	2908889	2.765513	3.009557
2.5	86265	0.082013	0.516858
3	37295	0.035457	0

Regression Output:	**no substrate**	
Constant		12.98035
Std Err of Y Est		0.527697
R Squared		0.988291
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-4.9854	
Std Err of Coef.	0.242677	

standard1

conc	area	regress
0	0	0
5	4945168	5401653
10	10527682	1.04E+07

Regression Output:	**standard1**	
Constant		0
Std Err of Y Est		201546.5
R Squared		0.998536
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	1040021	
Std Err of Coef.	18026.87	

standard2

conc	area	regress
0	0	0
5	4867794	5259221
10	10714155	1.05E+07

Regression Output:	**standard2**	
Constant		0
Std Err of Y Est		309450.1
R Squared		0.996672
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	1051844	
Std Err of Coef.	27678.05	

PHB **PHV**

acetate (PHB)

time	mass	area	%TSS
0.083333			
0.333	46.5	4131.13	6.515228
0.667	46.7	5694.04	8.941643
1	49	4098.74	6.134342
1.5	46	5515.46	8.793011
2	51	4504.7	6.477529
2.5	56	4945.4	6.476302
3	56.7	6155.02	7.960862

acetate (PHV)

time	mass	area	%TSS	PHV/PHB
0.083333				
0.333	48.5	1514.39	1.192133	0.182976
0.667	53.5	2286.55	1.631758	0.18249
1	42	1369.22	1.244666	0.202901
1.5	55	2226.71	1.545716	0.175789
2	44	1620.65	1.406259	0.217098
2.5	53	1759.4	1.267411	0.1957
3	57.5	2944.63	1.955202	0.245602

glucose (PHB)

time	mass	area	%TSS
0.083333			
0.333	42.5	2933.07	4.731704
0.667	52	4826.46	6.806738
1	52.5	2797.5	3.907729
1.5	43.5	2837.51	4.783677
2	55	4911.09	6.548305
2.5	50	3148.45	4.617857
3	52	4748.01	6.696101

glucose (PHV)

time	mass	area	%TSS	PHV/PHB
0.083333				
0.333	48.5	1324	1.042257	0.220271
0.667	53.5	2162.33	1.54311	0.226703
1	42	1193.41	1.084849	0.277616
1.5	55	1106.42	0.768044	0.160555
2	44	2221.32	1.927468	0.294346
2.5	53	1202.32	0.86611	0.187557
3	57.5	2070.51	1.374796	0.205313

no substrate (PHB)

time	mass	area	%TSS
0.083333			
0.333	48.5	2742.16	4.146338
0.667	53.5	4401.24	6.033023
1	42	3494.1	6.100982
1.5	55	4161.52	5.54885
2	44	3739.31	6.232359
2.5	53	2954.7	4.08838
3	57.5	5099.75	6.504213

no substrate (PHV)

time	mass	area	%TSS	PHV/PHB
0.083333				
0.333	48.5	1158.67	0.912109	0.219979
0.667	53.5	1821.89	1.300161	0.215507
1	42	1503.79	1.366994	0.224061
1.5	55	1607.73	1.116038	0.20113
2	44	1833.15	1.590648	0.255224
2.5	53	1052.97	0.758523	0.185531
3	57.5	2277.66	1.512341	0.232517

PHB standard

conc	area	regress
0	0	0
3.8	4252.37	5181.672
10.26	14334.7	13990.51

Regression Output:	**PHB standard**
Constant	0
Std Err of Y Est	700.7374
R Squared	0.990941
No. of Observations	3
Degrees of Freedom	2
X Coefficient(s)	1363.598
Std Err of Coef.	64.04636

PHV

PHV standard

conc	area	regress
0	0	0
1.2	2685.92	3143.059
3.24	8655.57	8486.259

Regression Output:	**PHV standard**
Constant	0
Std Err of Y Est	344.7044
R Squared	0.993946
No. of Observations	3
Degrees of Freedom	2
X Coefficient(s)	2619.216
Std Err of Coef.	99.76731

PHOSPHATE

acetate

time	area	conc	conc/10
0.083333	32533210	78.83062	7.883062
0.333	40502993	98.14206	9.814206
0.667	45124742	109.3409	10.93409
1	49544887	120.0513	12.00513
1.5	47167546	114.2908	11.42908
2	47567053	115.2589	11.52589
2.5	50073376	121.3319	12.13319
3	47181669	114.325	11.4325

glucose

time	area	conc	conc/10
0.083333	32108820	77.80229	7.780229
0.333	30735282	74.4741	7.44741
0.667	**33120480**	**80.25363**	8.025363
1	32013329	77.57091	7.757091
1.5	32744335	79.3422	7.93422
2	34297092	83.10465	8.310465
2.5	34889775	84.54077	8.454077
3	35446036	85.88864	8.588864

no substrate

time	area	conc	conc/10
0.083333	31592808	78.38927	7.838927
0.333	31194915	77.40201	7.740201
0.667	30756508	76.31421	7.631421
1	32479186	80.58859	8.058859
1.5	31859140	79.05011	7.905011
2	30609910	75.95047	7.595047
2.5	36818083	91.35442	9.135442
3	34188596	84.83004	8.483004

PO4-P standard 1

conc	area	regress
0	0	0
10	3521143	4126976
50	2.1E+07	2.1E+07

Regression Output:	**PO4-P standard 1**	
Constant		0
Std Err of Y Est		436872.4
R Squared		0.998453
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	412697.6	
Std Err of Coef.	8567.774	

PO4-P standard 2

conc	area	regress
0	0	0
10	3672404	4030246
50	2E+07	2E+07

Regression Output:	**PO4-P standard 2**	
Constant		0
Std Err of Y Est		258043.8
R Squared		0.999426
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	403024.6	
Std Err of Coef.	5060.654	

PHB batch 8/9 - duplicate

acetate duplicate

time	mass	area	%TSS
0.083333	54	1711.14	3.36666
0.333	57	1511.31	2.816995
0.667	51	2043.09	4.256227
1	52.5	3186.25	6.448044
1.5	53	2526.13	5.063925
2	53	2485.27	4.982016
2.5	41.5	1811.39	4.637365
3	54	1406.7	2.767676

glucose duplicate

time	mass	area	%TSS
0.083333	63	1268.06	2.138487
0.333	62	1803.26	3.090111
0.667	57.3	1338.09	2.481065
1	52	1683.43	3.439531
1.5	52	3810.48	7.785451
2	50	1664.23	3.536314
2.5	52	1370.35	2.799855
3	46.5	1406.7	3.214075

no substrate duplicate

time	mass	area	%TSS
0.083333	51.5	1925.06	3.971408
0.333	62.5	1500	2.549874
0.667	42	1553.94	3.930904
1	52	912.14	1.863655
1.5	46	1272.06	2.938037
2	57	1025.14	1.910802
2.5	55	1960.99	3.788089
3	50	1617.24	3.436465

PHV duplicate

acetate duplicate

time	mass	area	%TSS	PHV/PHB
0.083333	54	505.98	0.995513	0.295698
0.333	57	403.4	0.751915	0.266921
0.667	51	535.26	1.11507	0.261986
1	52.5	762.68	1.543443	0.239366
1.5	53	660.13	1.323308	0.261321
2	53	615.11	1.23306	0.247502
2.5	41.5	471.03	1.205891	0.260038
3	54	365.82	0.719749	0.260055

avg = 0.261611

glucose duplicate

time	mass	area	%TSS	PHV/PHB
0.083333	63	0	0	0
0.333	62	476.23	0.81608	0.264094
0.667	57.3	359.27	0.666153	0.268495
1	52	496.01	1.013432	0.294642
1.5	52	1351.72	2.761791	0.354737
2	50	433.08	0.920249	0.260228
2.5	52	348.01	0.711043	0.253957
3	46.5	385.99	0.881923	0.274394

avg = 0.246318

no substrate duplicate

time	mass	area	%TSS	PHV/PHB
0.083333	51.5	509.34	1.050771	0.264584
0.333	62.5	403.16	0.685338	0.268773
0.667	42	0	0	0
1	52	207.17	0.423283	0.227125
1.5	46	454.46	1.049652	0.357263
2	57	215.78	0.402202	0.210488
2.5	55	581.87	1.124011	0.296723
3	50	442.11	0.939437	0.273373

avg = 0.237291

PHB duplicate standard

conc	area
0	0
1.14	1491.1
4.18	3730.57
8.284	7842.36

Regression Output:	**PHB standard #2**	
Constant		0
Std Err of Y Est		269.7978
R Squared		0.993756
No. of Observations		4
Degrees of Freedom		3
X Coefficient(s)	941.223	
Std Err of Coef.	28.85965	

PHV duplicate standard

conc	area
0	0
0.36	650.34
1.32	1522.1
2.616	3364.6

Regression Output:	**PHV standard #2**	
Constant		0
Std Err of Y Est		144.7245
R Squared		0.990175
No. of Observations		4
Degrees of Freedom		3
X Coefficient(s)	1267.299	
Std Err of Coef.	49.02268	

Average PHB values

acetate average

time	%TSS 1	%TSS 2	mass 1	mass 2	average
0.083333	N/A	3.36666	N/A	54	3.36666
0.333	6.515228	2.816995	46.5	57	4.47852
0.667	8.941643	4.256227	46.7	51	6.495827
1	6.134342	6.448044	49	52.5	6.296602
1.5	8.793011	5.063925	46	53	6.796631
2	6.477529	4.982016	51	53	5.715393
2.5	6.476302	4.637365	56	41.5	5.693575
3	7.960862	2.767676	56.7	54	5.4276

glucose average

time	%TSS 1	%TSS 2	mass 1	mass 2	average
0.083333	N/A	2.138487	N/A	63	2.138487
0.333	4.731704	3.090111	42.5	62	3.757745
0.667	6.806738	2.481065	52	57.3	4.539025
1	3.907729	3.439531	52.5	52	3.67475
1.5	4.783677	7.785451	43.5	52	6.418151
2	6.548305	3.536314	55	50	5.114023
2.5	4.617857	2.799855	50	52	3.691033
3	6.696101	3.214075	52	46.5	5.052302

no substrate average

time	%TSS 1	%TSS 2	mass 1	mass 2	average
0.083333	N/A	3.971408	N/A	51.5	3.971408
0.333	4.146338	2.549874	48.5	62.5	3.247428
0.667	6.033023	3.930904	53.5	42	5.108531
1	6.100982	1.863655	42	52	3.756929
1.5	5.54885	2.938037	55	46	4.359767
2	6.232359	1.910802	44	57	3.793461
2.5	4.08838	3.788089	53	55	3.935454
3	6.504213	3.436465	57.5	50	5.077353

PHV average values

acetate average

time	%TSS 1	%TSS 2	mass	mass 2	average	PHV/PHB
0.083333	N/A	0.995513	46.7	54	0.995513	0.295698
0.333	1.192133	0.751915	48.5	57	0.95429	0.213082
0.667	1.631758	1.11507	53.5	51	1.379594	0.212382
1	1.244666	1.543443	42	52.5	1.410653	0.224034
1.5	1.545716	1.323308	55	53	1.436571	0.211365
2	1.406259	1.23306	44	53	1.311625	0.22949
2.5	1.267411	1.205891	53	41.5	1.240394	0.217859
3	1.955202	0.719749	57.5	54	1.356866	0.249994

glucose average

time	%TSS	%TSS 2	mass	mass 2	average	PHV/PHB
0.083333	N/A	0		63	0	0
0.333	1.042257	0.81608	48.5	62	0.915352	0.243591
0.667	1.54311	0.666153	53.5	57.3	1.089593	0.24005
1	1.084849	1.013432	42	52	1.045341	0.284466
1.5	0.768044	2.761791	55	52	1.736968	0.270634
2	1.927468	0.920249	44	50	1.391713	0.272137
2.5	0.86611	0.711043	53	52	0.789315	0.213847
3	1.374796	0.881923	57.5	46.5	1.154425	0.228495

no substrate average

time	%TSS	%TSS 2	mass	mass 2	average	PHV/PHB
0.083333	N/A	1.050771		51.5	1.050771	0.264584
0.333	0.912109	0.685338	48.5	62.5	0.784423	0.241552
0.667	1.300161	0	53.5	42	0.728362	0.142578
1	1.366994	0.423283	42	52	0.844941	0.224902
1.5	1.116038	1.049652	55	46	1.085803	0.249051
2	1.590648	0.402202	44	57	0.919941	0.242507
2.5	0.758523	1.124011	53	55	0.944651	0.240036
3	1.512341	0.939437	57.5	50	1.245874	0.245379

Batch 8/10

NO3-N

acetate

time	area	conc	regress
0.083333	12777463	12.14768	11.45746
0.333	10182717	9.680823	10.19647
0.667	9791121	9.308528	8.509537
1.416667	3204260	3.046326	4.723199
1.75	3277142	3.115616	3.039634
2	1331502	1.265874	1.77696
2.5	410250	0.390029	0
3	452126	0.429841	0

Regression Output:	**acetate**	
Constant		11.87835
Std Err of Y Est		1.072899
R Squared		0.956361
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-5.0507	
Std Err of Coef.	0.482492	

glucose

time	area	conc	regress
0.083333	11616560	11.16954	11.67859
0.333	11451672	11.011	10.43625
0.667	10110612	9.721543	8.774264
1.416667	3803608	3.657241	5.04392
1.75	2766898	2.660424	3.385252
2	2838940	2.729694	2.141251
2.5	169875	0.163338	0
3	172682	0.166037	0

Regression Output:	**glucose**	
Constant		12.09326
Std Err of Y Est		0.95305
R Squared		0.964233
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-4.976	
Std Err of Coef.	0.428595	

no substrate

time	area	conc	regress
0.083333	12743629	12.11551	11.30179
0.333	10414606	9.901282	10.37546
0.667	9451492	8.985639	9.136236
1.416667	5203127	4.946671	6.354783
1.75	5117530	4.865293	5.118032
2	8268351	7.860814	4.190469
2.5	1556521	1.479802	2.335342
3	68238	0.064875	0

Regression output:	**no substrate**	
Constant		11.61097
Std Err of Y Est		1.763588
R Squared		0.847581
No. of Observations		8
Degrees of Freedom		6
X Coefficient(s)	-3.71025	
Std Err of Coef.	0.642328	

standard1

conc	area	regress
0	0	0
5	4945168	5401653
10	10527682	1.04E+07

Regression Output:	**standard1**	
Constant		0
Std Err of Y Est		201546.5
R Squared		0.998536
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	1040021	
Std Err of Coef.	18026.87	

standard2

conc	area	regress
0	0	0
5	4867794	5259221
10	10714155	1.05E+07

Regression Output:	**standard2**	
Constant		0
Std Err of Y Est		309450.1
R Squared		0.996672
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	1051844	
Std Err of Coef.	27678.05	

no substrate-excluding bad data point at 2 hrs

time	area	conc	regress
0.083333	12743629	12.11551	11.30312
0.333	10414606	9.901282	10.30184
0.667	9451492	8.985639	8.962334
1.416667	5203127	4.946671	5.955808
1.75	5117530	4.865293	4.618981
2			3.61636
2.5	1556521	1.479802	1.611118
3	68238	0.064875	0

Regression Output:	**no substrate2**	
Constant		11.63733
Std Err of Y Est		0.652367
R Squared		0.982204
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-4.01048	
Std Err of Coef.	0.241422	

PHB

acetate

time	mass	area	%TSS
0.0833	49.5	3723.5	4.937371
0.333	40	2566.84	4.212
0.667	46	4781.05	6.822052
1.416667	42	3631.16	5.674736
1.75	44.5	2198.23	3.242371
2	40	2613.97	4.289337
2.5	47	3564.54	4.978004
3	43.5	2968.3	4.478867

glucose

time	mass	area	%TSS
0.0833	39	3719.64	6.260167
0.333	46.5	2713.34	3.830018
0.667	40.5	3397.1	5.505578
1.416667	45.5	1926.01	2.778413
1.75	45.7	3857.82	5.540837
2	56.5	2311.42	2.685221
2.5	43.5	1767.35	2.666754
3	50	2314.83	3.038776

no substrate

time	mass	area	%TSS
0.0833			
0.333	40	1616.93	2.653266
0.667	49.2	1252.65	1.671145
1.416667	40	3399.53	5.578385
1.75	43.5	3108.36	4.690203
2	39	2910.21	4.897893
2.5	47.5	2234.17	3.087253
3	44.3	3857.23	5.715068

PHV

acetate

time	mass	area	%TSS	PHV/PHB
0.0833	49.5	886.73	0.504878	0.102257
0.333	40	622.08	0.438315	0.104063
0.667	46	1345.71	0.824507	0.120859
1.416667	42	932.5	0.625749	0.110269
1.75	44.5	633.85	0.401446	0.123812
2	40	643.67	0.453528	0.105734
2.5	47	1117.4	0.670056	0.134603
3	43.5	691.36	0.447936	0.100011

glucose

time	mass	area	%TSS	PHV/PHB
0.0833	39	1091.3	0.788642	0.125978
0.333	46.5	789.11	0.478283	0.124878
0.667	40.5	1067.36	0.742774	0.134913
1.416667	45.5	497.7	0.308288	0.110958
1.75	45.7	1277.46	0.787828	0.142186
2	56.5	607.53	0.303054	0.11286
2.5	43.5	467.45	0.302863	0.11357
3	50	613.75	0.345957	0.113847

no substrate

time	mass	area	%TSS	PHV/PHB
0.0833				
0.333	40	418.67	0.294993	0.111181
0.667	49.2	355.16	0.203451	0.121743
1.416667	40	995.5	0.701426	0.12574
1.75	43.5	912.06	0.590928	0.125992
2	39	799.41	0.577704	0.11795
2.5	47.5	591.65	0.351052	0.11371
3	44.3	1319.53	0.839491	0.146891

PHB standard

conc	PHB	area	regress
0	0	0	0
5	3.8	5732.33	5789.406
6.5	4.94	6025.94	7526.228
9.5	7.22	16481.5	10999.87
13.5	10.26	18142.5	15631.4
14	10.64	24778.7	16210.34

Regression Output:	**PHB standard**	
Constant		0
Std Err of Y Est		2842.409
R Squared		0.908183
No. of Observations		6
Degrees of Freedom		5
X Coefficient(s)	1523.528	
Std Err of Coef.	122.802	

PHV standard

conc	PHV	area	regress
0	0	0	0
5	1.2	3562.06	4257.755
6.5	1.56	3463.59	5535.082
9.5	2.28	9144.66	8089.735
13.5	3.24	9965.79	11495.94
14	3.36	13891.6	11921.71

Regression Output:	**PHV standard**	
Constant		0
Std Err of Y Est		1556.261
R Squared		0.909336
No. of Observations		6
Degrees of Freedom		5
X Coefficient(s)	3548.129	
Std Err of Coef.	280.1497	

PHOSPHATE

acetate

time	area	conc	conc/10
0.083333	40685347	100.95	10.095
0.333	49686463	123.2839	12.32839
0.667	51242957	127.146	12.7146
1.416667	57756962	143.3088	14.33088
1.75	56853428	141.0669	14.10669
2	58581728	145.3552	14.53552
2.5	56972256	141.3617	14.13617
3	56441563	140.0449	14.00449

glucose

time	area	conc	
0.083333	42709035	105.9713	10.59713
0.333	42748978	106.0704	10.60704
0.667	***25438673***	63.1194	6.31194
1.416667	44160925	109.5738	10.95738
1.75	43697211	108.4232	10.84232
2	45375150	112.5865	11.25865
2.5	45800563	113.6421	11.36421
3	45353484	112.5328	11.25328

no substrate

time	area	conc	
0.083333	44377405	117.7549	11.77549
0.333	44197751	117.2782	11.72782
0.667	41229628	109.4023	10.94023
1.416667	44679942	118.5577	11.85577
1.75	43682838	115.9119	11.59119
2	***44540792***	118.1885	11.81885
2.5	45398746	120.465	12.0465
3	45381162	120.4184	12.04184

PO4-P standard 1

conc	area	regress
0	0	0
10	3672404	4030246
50	20222800	2.02E+07

Regression Output:	**PO4-P standard**	
Constant		0
Std Err of Y Est		258043.8
R Squared		0.999426
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	403024.6	
Std Err of Coef.	5060.654	

Phosphate standard 2

conc	area	regress
0	0	0
10	3192408	0
50	18958365	0

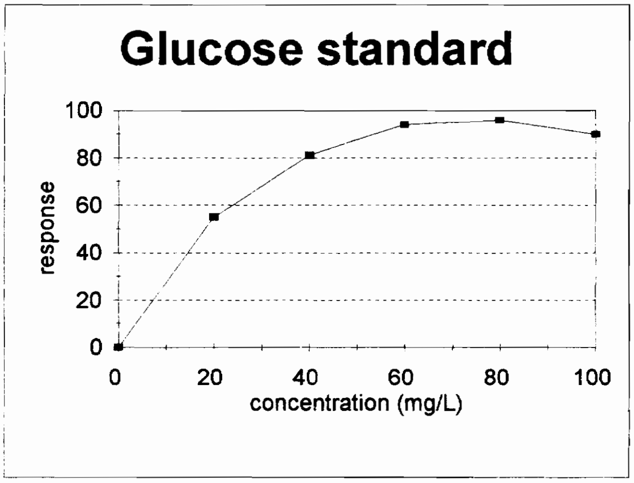
Regression Output:	**PO4-P standard**	
Constant		0
Std Err of Y Est		415515.5
R Squared		0.998324
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	376862.4	
Std Err of Coef.	8148.93	

glucose analysis

time	response	conc
0.083333	34	12.36364
0.333	22	8
0.667	11	4
1.416667	4	1.454545
1.75	0	0
2	0	0
2.5	0	0
3	0	0

standard

conc	response
100	90
80	96
60	94
40	81
20	55
0	0



Batch 10/2 - 8 degrees C

NO3-N

acetate

time	area	conc	regress
0.083333	13713516	15.58999	13.62288
0.333	10338217	11.75283	12.07208
0.667	8167065	9.284595	9.997429
1	5847767	6.647939	7.928995
1.5	3745168	4.257633	4.823236
2	1038873	1.181026	1.717478
2.5	52605	0.059803	0
3	***573581***	0.652066	0

Regression Output:	**acetate**	
Constant		14.14051
Std Err of Y Est		1.328554
R Squared		0.953857
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-6.21152	
Std Err of Coef.	0.610973	

glucose

time	area	conc	regress
0.083333	13323841	15.14699	13.34231
0.333	11270272	12.81243	12.20962
0.667	7501355	8.527794	10.69432
1	6525638	7.418566	9.183552
1.5	10507804	***11.94563***	6.915139
2	5664009	6.439037	4.646726
2.5	1952720	2.219918	2.378313
3	502513	0.571274	0

Regression Output:	**glucose**	
Constant		13.72038
Std Err of Y Est		1.713406
R Squared		0.915159
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-4.53683	
Std Err of Coef.	0.617762	

data point at 1.5 hours was ignored in regression

no substrate

time	area	conc	regress
0.083333	12680170	14.41525	11.64585
0.333	9170952	10.42585	10.50062
0.667	5283269	6.006199	8.968557
1	6630269	7.537514	7.441076
1.5	2799169	3.18219	5.147561
2	3777301	4.294163	2.854046
2.5	1105781	1.257089	0.560531
3	54443	0.061893	0

Regressioı Regressioı	**no substrate**	
Constant		12.02811
Std Err of Y Est		2.139243
R Squared		0.813013
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-4.58703	
Std Err of Coef.	0.983792	

standard

conc	area	regress
0	0	0
5	4163036	4584078
10	8913933	8796362

Regression Output:	**standard**	
Constant		0
Std Err of Y Est		185898
R Squared		0.998263
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	879636.1	
Std Err of Coef.	16627.22	

PHB

acetate

time	mass	area	%TSS
0.083333	54.5	2585.18	4.462712
0.333	55	1381.4	2.362987
0.667	51	2720.5	5.018607
1	55.5	948.99	1.608693
1.5	52	2543.94	4.602652
2	58.5	2489.34	4.003437
2.5	58	2947.17	4.780593
3	57.5	2597.12	4.249411

PHB duplicate

mass	area	%TSS	average
55	1307.24	2.245287	3.348937
57	1489.64	2.468799	2.416838
52.5	1467.72	2.640969	3.812558
53.5	2397.49	4.233332	2.896933
51.5	1923.66	3.528584	4.068212
56	2065.4	3.484139	3.749457
63.7	2742.23	4.066715	4.406936
53	1704.77	3.03857	3.668646

glucose

time	mass	area	%TSS
0.083333	56	1599.93	2.687927
0.333	50.5	1349.81	2.514698
0.667	51.5	1533.02	2.800562
1	54	2198.31	3.830009
1.5	58	1146.12	1.859117
2	59	2058.48	3.282458
2.5	59	1815.72	2.895353
3	56	1155.79	1.94176

mass	area	%TSS	average
59.5	1414.13	2.245183	2.459847
52.5	1057.61	1.90303	2.202925
58.5	1991.56	3.216006	3.021503
53	1770.65	3.155994	3.496151
59.5	853.24	1.35467	1.603674
57	1952.41	3.235754	3.259509
57.5	1409.66	2.315932	2.609373
52.5	691.39	1.244065	1.604166

no substrate

time	mass	area	%TSS
0.083333	54.5	2314.39	3.995256
0.333	51.5	1347.76	2.462124
0.667	50	1307.595	2.460412
1	55.5	1267.43	2.148501
1.5	54.5	2615.58	4.515191
2	50.7	1580.82	2.933452
2.5	54.5	2524.95	4.358739
3	65.5	2253.97	3.237512

mass	area	%TSS	average
53	952.6	1.697907	2.86261
59	1281.85	2.052412	2.243364
53	2008.95	3.580738	3.036891
55	1497.93	2.572813	2.359697
50.5	1873.61	3.504831	4.029256
57.5	1219.67	2.003797	2.439412
55	1560.58	2.680419	3.515747
65.5	1951.23	2.814145	3.025829

PHB standard

conc	PHB	area	regress
0	0	0	0
3.5	2.66	3809.21	2827.334
5.5	4.18	4490.69	4442.953
8.2	6.232	7430.3	6624.039
10.9	8.284	7859.21	8805.124

Regression Output:	**PHB standard**	
Constant		0
Std Err of Y Est		792.3339
R Squared		0.937782
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	1062.907	
Std Err of Coef.	68.96107	

PHB standard-duplicates

conc	PHB	area	regress
0	0	0	0
3.5	2.66	3000.44	2827.334
5.5	4.18	3665.92	4442.953
8.2	6.232	7143.55	6624.039
10.9	8.284	8681.72	8805.124

Regression Output:	**PHB standard duplicate**	
Constant		0
Std Err of Y Est		478.6403
R Squared		0.980776
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	1058.573	
Std Err of Coef.	41.65864	

PHV

acetate / **PHV duplicate**

time	mass	area	%TSS	PHV/PHB	mass	area	%TSS	average
0.083333	54.5	1497.77	0.81649	0.246	55	679.35	0.829928	0.823239
0.333	55	652.49	0.352463	0.258	57	749.76	0.883806	0.622878
0.667	51	1526.48	0.889249	0.225	52.5	647.42	0.828583	0.858476
1	55.5	335.8	0.179759	0.275	53.5	1142.27	1.434578	0.795656
1.5	52	1139.86	0.651254	0.212	51.5	827.13	1.079135	0.864161
2	58.5	1084.16	0.550605	0.224	56	954.52	1.145266	0.841443
2.5	58	1183.18	0.606073	0.224	63.7	1262.11	1.331273	0.985656
3	57.5	1191.8	0.615797	0.206	53	713.55	0.904603	0.754319

avg = 0.234

glucose

time	mass	area	%TSS	PHV/PHB	mass	area	%TSS	average
0.083333	56	919.73	0.487949	0.279	59.5	774.22	0.874292	0.686974
0.333	50.5	791.86	0.465864	0.258	52.5	522.75	0.669028	0.569418
0.667	51.5	971.32	0.560347	0.268	58.5	897.99	1.031395	0.810859
1	54	1185.68	0.652343	0.263	53	942.02	1.194245	0.920762
1.5	58	1146.12	0.58709	0.327	59.5	410.1	0.463108	0.524307
2	59	1278.74	0.643921	0.299	57	1119.72	1.319909	0.976087
2.5	59	838.19	0.422078	0.250	57.5	762.61	0.891136	0.653587
3	56	617.01	0.327346	0.226	52.5	311.6	0.398793	0.361917

avg = 0.271

no substrate

time	mass	area	%TSS	PHV/PHB	mass	area	%TSS	average
0.083333	54.5	1449.44	0.790144	0.236	53	439.89	0.55767	0.675529
0.333	51.5	681.63	0.393227	0.261	59	662.11	0.754028	0.585872
0.667	50	668.37	0.397145	0.286	53	1035.67	1.31297	0.868395
1	55.5	655.11	0.35069	0.281	55	799.91	0.97721	0.662532
1.5	54.5	1536.98	0.837865	0.259	50.5	951.06	1.265394	1.043486
2	50.7	802.34	0.470168	0.243	57.5	598.9	0.699835	0.592218
2.5	54.5	1704.94	0.929426	0.274	55	814.56	0.995107	0.962416
3	65.5	1304	0.591478	0.260	65.5	957.25	0.981959	0.786718

avg = 0.262419

PHV standard

conc	PHV	area	regress
0	0	0	0
3.5	0.84	3809.21	2827.334
5.5	1.32	4490.69	4442.953
8.2	1.968	7430.3	6624.039
10.9	2.616	7859.21	8805.124

Regression Output:	**PHV standard**	
Constant		0
Std Err of Y Est		792.3339
R Squared		0.937782
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	3365.873	
Std Err of Coef.	218.3767	

PHV standard - duplicates

conc	PHV	area	regress
0	0	0	0
3.5	0.84	1502.3	1250.173
5.5	1.32	1448.22	1964.557
8.2	1.968	3520.93	2928.976
10.9	2.616	3627.65	3893.395

Regression Output:	**PHV standard - duplicate**	
Constant		0
Std Err of Y Est		433.3596
R Squared		0.921031
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	1488.301	
Std Err of Coef.	119.4391	

PHOSPHATE

acetate

time	area	conc	conc/10
0.083333	17168103	47.11744	4.711744
0.333	23772446	65.2429	6.52429
0.667	32008581	87.84677	8.784677
1	33972874	93.23773	9.323773
1.5	33551518	92.08133	9.208133
2	42715234	117.2309	11.72309
2.5	38509331	105.6879	10.56879
3	31032202	85.16712	8.516712

glucose

time	area	conc	
0.083333	19790417	54.31432	5.431432
0.333	25463523	69.88402	6.988402
0.667	25990536	71.33039	7.133039
1	27412589	75.23318	7.523318
1.5	***4040571***	11.08925	1.108925
2	15336051	42.08942	4.208942
2.5	19055520	52.29741	5.229741
3	10723447	29.43024	2.943024

no substrate

time	area	conc	
0.083333	14090825	38.67193	3.867193
0.333	20432095	56.07539	5.607539
0.667	13275888	36.43535	3.643535
1	12464922	34.20968	3.420968
1.5	11031263	30.27503	3.027503
2	***8479435***	23.2716	2.32716
2.5	24512983	67.27529	6.727529
3	26041861	71.47125	7.147125

PO4-P standard 1

conc	area	regress
0	0	0
10	3587338	3643683
50	18229685	1.8E+07

Regression Output:	**PO4-P standard**	
Constant		0
Std Err of Y Est		40631.1
R Squared		0.999982
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	364368.3	
Std Err of Coef.	796.8414	

ACETATE

time	area	conc	acetate/10
0.083333	285009	68.08137	6.808137
0.333	261112	59.95315	5.995315
0.667	230473	49.53174	4.953174
1	189556	35.61443	3.561443
1.5	192790	36.71442	3.671442
2	94545	3.297825	0
2.5	91050	2.109052	0
3	95439	3.601906	0

this data point may be incorrect-a double injection may have occurred

ACETATE standard

conc	area	regress
0	95000	84849.38
12.5	136977	121599.5
25	157578	158349.5
50	185795	231849.7
100	400148	378850

Regression Output:		**ACETATE standard**
Constant		84849.38
Std Err of Y Est		31170.17
R Squared		0.948808
No. of Observations		5
Degrees of Freedom		3
X Coefficient(s)	2940.006	
Std Err of Coef.	394.2749	

Batch 10/3

NO3-N

acetate

time	area	conc	regress
0.083333	9172058	11.75194	12.00934
0.333	8749695	11.21078	10.55215
0.667	6755310	8.655421	8.602736
1	4139501	5.303846	6.659161
1.5	3981868	5.101874	3.740879
2	283312	0.363001	0.822598
2.5	94432	0.120994	0
3	67686	0.086724	0

Regression Output:	**acetate**	
Constant		12.49572
Std Err of Y Est		1.049195
R Squared		0.952935
No. of Observations		6
Degrees of Freedom		4
X Coefficient(s)	-5.83656	
Std Err of Coef.	0.648551	

glucose

time	area	conc	regress
0.083333	8155269	10.44916	11.31731
0.333	8729941	11.18547	10.09463
0.667	5593229	7.166473	8.458947
1	6982705	8.946777	6.828162
1.5	2963310	3.79682	4.379536
2	739171	0.947083	1.93091
2.5	***8321750***	10.66246	0
3	65558	0.083998	0

Regression Output:	**glucose**	
Constant		11.72541
Std Err of Y Est		1.391269
R Squared		0.921367
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-4.89725	
Std Err of Coef.	0.639814	

this glucose regression ignores data point at time 2.5 h

no substrate

time	area	conc	regress
0.083333	9976982	12.78327	11.18673
0.333	7292514	9.343728	9.992674
0.667	6549910	8.392246	8.39529
1	4535565	5.811313	6.802689
1.5	2931195	3.755672	4.411396
2	1102587	1.412719	2.020103
2.5	732656	0.938735	0
3	51972	0.066591	0

Regression output:	**no substrate**	
Constant		11.58527
Std Err of Y Est		1.137341
R Squared		0.943573
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-4.78259	
Std Err of Coef.	0.523039	

standard

conc	area	regress
0	0	0
5	3601258	4140398
10	7955266	7804717

Regression Output:	**standard**	
Constant		0
Std Err of Y Est		238040.5
R Squared		0.996429
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	780471.6	
Std Err of Coef.	21290.99	

PHB

acetate

time	mass	area	%TSS
0.083333	55	1615.62	2.616372
0.333	58	1372.89	2.108292
0.667	52	1498.56	2.56681
1	53.5	1544.7	2.571659
1.5	54	1146.68	1.891348
2	52	1528.6	2.618264
2.5	51	2435.63	4.253673
3	52.5	1815.41	3.079913

glucose

time	mass	area	%TSS
0.083333	55.5	1120.22	1.797767
0.333	52	1487.94	2.54862
0.667	55.5	1208.1	1.938799
1	55	950.66	1.539521
1.5	58	620.5	0.952877
2	52.5	1334.3	2.263691
2.5	61	967.79	1.413105
3	51.5	1636.41	2.83014

no substrate

time	mass	area	%TSS
0.083333	56	1837.01	2.921773
0.333	56	1579.86	2.512775
0.667	50	1482.94	2.641658
1	54.5	1083.22	1.770285
1.5	52	1764.95	3.023097
2	53.5	1659.76	2.763214
2.5	52	1842.4	3.155757
3	52	1519.34	2.602403

PHV

acetate

time	mass	area	%TSS	PHV/PHB
0.083333	55	821.59	0.934534	0.357187
0.333	58	575.86	0.621143	0.294619
0.667	52	637.93	0.76749	0.299005
1	53.5	526.23	0.615354	0.239283
1.5	54	408.67	0.473458	0.250329
2	52	591.36	0.711462	0.27173
2.5	51	1044.98	1.28186	0.301354
3	52.5	713.89	0.850697	0.276208
			avg =	0.286214

glucose

time	mass	area	%TSS	PHV/PHB
0.083333	55.5	565.53	0.637479	0.354595
0.333	52	823.85	0.991169	0.388904
0.667	55.5	654.43	0.737689	0.380487
1	55	457.4	0.520279	0.337949
1.5	58	265.06	0.285903	0.300042
2	52.5	675.18	0.804569	0.355423
2.5	61	455.27	0.46692	0.330421
3	51.5	723.85	0.879314	0.310696
			avg =	0.344815

no substrate

time	mass	area	%TSS	PHV/PHB
0.083333	56	936.28	1.045973	0.357993
0.333	56	780.35	0.871775	0.346937
0.667	50	683.32	0.854982	0.323654
1	54.5	529.8	0.608161	0.343538
1.5	52	971.13	1.168361	0.386478
2	53.5	857.73	1.002997	0.362982
2.5	52	829.77	0.998291	0.31634
3	52	628.25	0.755844	0.290441
			avg =	0.341045

PHB standard

conc	PHB	area	regress
0	0	0	0
3.5	2.66	3363.06	2986.473
5.5	4.18	4428.3	4693.03
8.2	6.232	7533.53	6996.88
10.9	8.284	8909.67	9300.731

Regression Output:	**PHB standard**	
Constant		0
Std Err of Y Est		403.9867
R Squared		0.986837
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	1122.734	
Std Err of Coef.	35.16113	

PHV standard

conc	PHV	area	regress
0	0	0	0
3.5	0.84	1700.95	1342.692
5.5	1.32	1774.59	2109.945
8.2	1.968	3750.44	3145.736
10.9	2.616	3780.79	4181.527

Regression Output:	**PHV standard**	
Constant		0
Std Err of Y Est		437.9123
R Squared		0.924596
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	1598.443	
Std Err of Coef.	120.6939	

PHOSPHATE

acetate

time	area	conc	conc/10
0.083333	18071057	57.80222	5.780222
0.333	24166333	77.29862	7.729862
0.667	23515628	75.21727	7.521727
1	26209262	83.83315	8.383315
1.5	21688945	69.37443	6.937443
2	45311737	144.9345	14.49345
2.5	34249676	109.5513	10.95513
3	30454955	97.41346	9.741346

glucose

time	area	conc	
0.083333	19281963	61.67544	6.167544
0.333	28399255	90.83808	9.083808
0.667	***9716816***	31.08028	3.108028
1	11033011	35.29027	3.529027
1.5	19646259	62.84068	6.284068
2	31388595	100.3998	10.03998
2.5	***3479280***	11.12885	1.112885
3	25963775	83.04793	8.304793

no substrate

time	area	conc	
0.083333	***8005733***	25.6072	2.56072
0.333	20199204	64.60933	6.460933
0.667	29702115	95.00542	9.500542
1	23235026	74.31973	7.431973
1.5	20888896	66.81539	6.681539
2	20949868	67.01041	6.701041
2.5	20297432	64.92352	6.492352
3	30550749	97.71986	9.771986

PO4-P standard 1

conc	area	regress
0	0	0
10	2890265	3126360
50	15679020	1.6E+07

Regression Output:	**PO4-P standard**	
Constant		0
Std Err of Y Est		170250.7
R Squared		0.999584
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	312636	
Std Err of Coef.	3338.89	

ACETATE

time	area	conc	acetate/10
0.083333	197396	68.32889	6.832889
0.333	121534	25.76556	2.576556
0.667	98360	12.7635	1.27635
1	116612	23.00401	2.300401
1.5	87140	6.468378	0.646838
2	88380	7.164095	0.71641
2.5	77690	1.166337	0.116634
3	85004	5.269948	0.526995

ACETATE standard

conc	area	regress
0	66681	75611.2
25	124621	120169.5
50	175911	164727.8
100	247140	253844.5

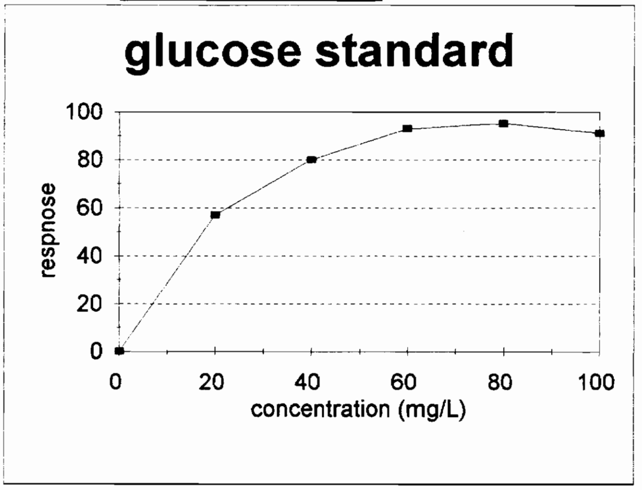
Regression Output:	**acetate standard**	
Constant		75611.2
Std Err of Y Est		11609.85
R Squared		0.98472
No. of Observations		4
Degrees of Freedom		2
X Coefficient(s)	1782.333	
Std Err of Coef.	156.9938	

glucose analysis

time	response	conc
0.083333	30	10.52632
0.333	25	8.77193
0.667	15	5.263158
1	17	5.964912
1.5	11	3.859649
2	5	1.754386
2.5	0	0
3	0	0

standard

conc	response
100	91
80	95
60	93
40	80
20	57
0	0



Batch 10/4

NO3-N

acetate

time	area	conc	regress
0.083333	9808819	12.56781	12.53014
0.333	8083914	10.35773	10.97269
0.667	7287704	9.337565	8.889148
1.416667	3787585	4.852944	4.21262
1.75	1607738	2.059957	2.133239
2	105782	0.135536	0.573703
2.5	40139	0.051429	0
3	43586	0.055846	0

Regression Output:	**acetate**	
Constant		13.04999
Std Err of Y Est		0.544987
R Squared		0.990315
No. of Observations		6
Degrees of Freedom		4
X Coefficient(s)	-6.23814	
Std Err of Coef.	0.308452	

glucose

time	area	conc	regress
0.083333	9734582	12.47269	11.70897
0.333	8378520	10.7352	10.52676
0.667	5797940	7.428765	8.945227
1.416667	4958468	6.353169	5.395451
1.75	1874285	2.401478	3.817071
2	2845809	3.646268	2.633287
2.5	198951	0.254911	0.265717
3	35982	0.046103	0

Regressio Regressio	**glucose**	
Constant		12.10356
Std Err of Y Est		1.172498
R Squared		0.94162
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-4.73514	
Std Err of Coef.	0.527283	

no substrate

time	area	conc	regress
0.083333	9451929	12.11054	10.92711
0.333	6874936	8.808695	9.822254
0.667	5623094	7.204739	8.344194
1.416667	4872680	6.243251	5.026672
1.75	3197149	4.096432	3.551563
2	1437731	1.842131	2.445231
2.5	34191	0.043808	0
3	67656	0.086686	0

Regression output:	**no substrate**	
Constant		11.29589
Std Err of Y Est		1.086504
R Squared		0.942548
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-4.42533	
Std Err of Coef.	0.488611	

standard

conc	area	regress
0	0	0
5	3601258	4140398
10	7955266	7804717

Regression Output:	**standard**	
Constant		0
Std Err of Y Est		238040.5
R Squared		0.996429
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	780471.6	
Std Err of Coef.	21290.99	

Statistical Evaluation of the PHB method used by Hart/Seyfried

vial	mass	area	%TSS
1	35.5	4627.09	11.58
2	32.5	3845.89	10.52
16	36.0	4824.42	11.91
33	25.5	2815.87	9.81
34	35.5	4309.16	10.79
35	34.5	3139.34	8.09
36	32.0	3047.66	8.46
37	30.5	3805.86	11.09
38	35.0	4288.57	10.89
39	36.0	3978.53	9.82

Column 1	***95 %***
Mean	10.30
Standard Error	0.40
Median	10.65
Mode	NA
Standard Deviation	1.26
Variance	1.58
Kurtosis	-0.38
Skewness	-0.70
Range	3.82
Minimum	8.09
Maximum	11.91
Sum	102.95
Count	10.00
Confidence Level(0.	0.78

Column 1	***99 %***
Mean	10.30
Standard Error	0.40
Median	10.65
Mode	NA
Standard Deviation	1.26
Variance	1.58
Kurtosis	-0.38
Skewness	-0.70
Range	3.82
Minimum	8.09
Maximum	11.91
Sum	102.95
Count	10.00
Confidence Level(0.	1.02

PHB standard

conc	area	regress
0.00	0.00	0.00
5.00	5065.59	5626.77
6.00	5800.67	6752.13
8.30	6706.38	9340.44
13.20	17156.00	14854.68

Regression Output: **PHB standard**		
Constant		0.00
Std Err of Y Est		1834.02
R Squared		0.91
No. of Observations		5.00
Degrees of Freedom		4.00
X Coefficient(s)	1125.35	
Std Err of Coef.	105.17	

Therefore, the Hart/Seyfried/Braunegg PHB method has a 95% confidence interval of +/- 0.78 %TSS and a 99 % interval of +/- 1.02 %TSS statistical analysis of method by Comeau, etc.

sample	area	mass	%TSS
1	2247.34	28.5	8.314522
2	2822.71	30	9.921067
3	3534.09	37	10.07139
4	3392.92	32	11.17988
5	5152.53	31.5	17.24739
6	2887.29	35	8.698327
7	2816.18	32.5	9.136722
8	3333.63	35	10.04298
9	4376.16	36.5	12.64194
10	4252.86	37.5	11.95813

Column 1	
Mean	10.92123
Std Err	0.826893
Median	10.05718
Mode	NA
Std Dev	2.614867
Variance	6.837527
Kurtosis	3.570739
Skewness	1.730205
Range	8.932866
Minimum	8.314522
Maximum	17.24739
Sum	109.2123
Count	10
Conf Lev(0	1.620681

PHB std

conc	area	regress
0	0	0
3.2	2464.46	3034.846
4.6	4105.22	4362.591
6	5660.05	5690.336
10.2	9889.13	9673.571
11.3	10804.6	10716.8

Regression Output:	PHB standard	
Constant		0
Std Err of Y Est		298.8885
R Squared		0.994972
No. of Observations		6
Degrees of Freedom		5
X Coefficient(s)	948.3893	
Std Err of Coef.	17.28141	

statistical evaluation of modification of method outlined by Comeau

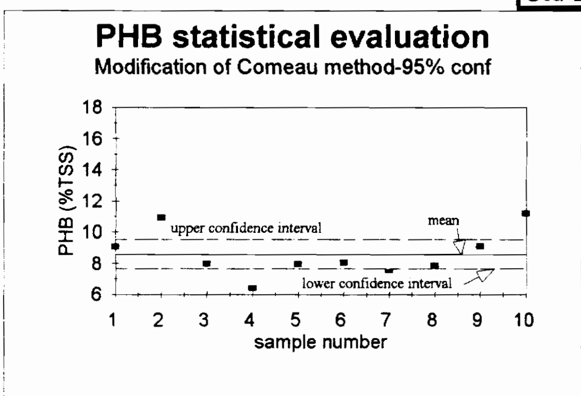
sample	mass	area	%TSS
1	22.5	1976	9.071679
2	25.9	2736.99	10.91583
3	27.5	2129.96	8.00059
4	31	1923.21	6.40838
5	28.1	2166.99	7.965881
6	23	1794.75	8.060452
7	25.7	1843.75	7.410579
8	27.1	2060.9	7.855446
9	27.1	2387.7	9.101096
10	22.2	2408.09	11.20477

Column 1	
Mean	8.59947
Std Error	0.476635
Median	8.030521
Mode	NA
Std Dev	1.507251
Variance	2.271807
Kurtosis	-0.08086
Skewness	0.693858
Range	4.796391
Minimum	6.40838
Maximum	11.20477
Sum	85.9947
Count	10
Conf Lev((	0.934187

PHB standard

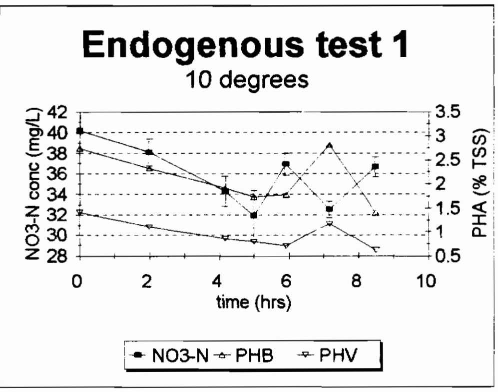
conc	area	regress
0	0	0
3.2	2452.59	3097.895
4.3	2923.09	4162.797
6.3	5059.37	6098.981
7.4	6741.77	7163.883
16.4	16917.5	15876.71

Regression Output:	**PHB standard**	
Constant		0
Std Err of Y Est		926.8764
R Squared		0.975878
No. of Observations		6
Degrees of Freedom		5
X Coefficient(s)	968.0923	
Std Err of Coef.	46.80597	



Endogenous test #1
MLSS = 1900

time	area	conc
0.00	3.5E+07	39.94048
2.00	3.4E+07	39.38113
4.17	2.9E+07	32.85814
5.00	3E+07	34.37606
5.92	3.1E+07	35.86808
7.17	2.8E+07	31.76231
8.50	3.1E+07	35.70626



conc	area
0	0
5	4312724
10	8760293

Regression Output:		
Constant		0
Std Err of Y Est		42641.73
R Squared		0.999905
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	873332.4	
Std Err of Coef.	3813.993	

time	area	conc	avg conc
0.00	3.5E+07	40.41547	40.17798
2.00	3.2E+07	36.7875	38.08432
4.17	3.1E+07	35.74753	34.30283
5.00	2.6E+07	29.59708	31.98657
5.92	3.3E+07	38.01007	36.93907
7.17	2.9E+07	33.35169	32.557
8.50	3.3E+07	37.63344	36.66985

0	0
5	4172981
10	8793647

Regression Output:		
Constant		0
Std Err of Y Est		141570.4
R Squared		0.998964
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	870411	
Std Err of Coef.	12662.44	

PHB data

time	mass 1	mass 2	area 1	area 2	%TSS 1	% TSS 2	avg %TSS
0.00	78.5	86	2962.27	3645.08	2.580013	2.897848	2.746176
2.00	86.5	78	2187.76	3418.29	1.729221	2.996272	2.330011
4.17	85.5	78	2502.77	2065.29	2.001344	1.810312	1.91021
5.00	90	99	2373.68	2432.41	1.803211	1.679842	1.73859
5.92	101	90.5	2334.51	2644.68	1.580307	1.997982	1.777694
7.17	86.5	96	5042.1	2486.34	3.985312	1.770746	2.82039
8.50	89.5	80	1924.44	1559.87	1.470105	1.333108	1.405446
14.00	75.5	84	1889.39	1810.82	1.710967	1.473883	1.586108
18.50	74.5	70	3403.44	2414.71	3.123409	2.358489	2.752859
21.75	73	65.5	4616.19	2106.83	4.323424	2.199152	3.318804

PHV data

time	mass 1	mass 2	area 1	area 2	% TSS 1	% TSS 2	avg %TSS	PHV/PHB
0.00	78.5	86	2280.64	2980.98	1.288063	1.536776	1.418089	0.516387
2.00	86.5	78	1658.28	2430.87	0.849947	1.38171	1.10209	0.472998
4.17	85.5	78	1753.79	1400.2	0.909413	0.795876	0.855249	0.447725
5.00	90	99	1769.38	1624.1	0.871623	0.727323	0.796037	0.457864
5.92	101	90.5	1382.08	1679.43	0.606683	0.822741	0.708789	0.398713
7.17	86.5	96	3147.75	1672.26	1.61337	0.772294	1.170941	0.41517
8.50	89.5	80	1290.38	1083.16	0.639211	0.600278	0.620836	0.441736
14.00	75.5	84	1514.11	1332.5	0.88912	0.703295	0.791256	0.498867
18.50	74.5	70	3315.52	1899.92	1.973083	1.203337	1.600195	0.581285
21.75	73	65.5	4405.7	1775.74	2.675728	1.201954	1.978745	0.596222
								0.482697

PHB standard	
0	0
1.748	3315.13
3.99	5604.25
5.32	6541.82
9.31	14282.1

Regression Output:	**PHB standard**	
Constant		0
Std Err of Y Est		807.3407
R Squared		0.976771
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	1462.625	
Std Err of Coef.	69.75556	

PHV standard

0	0
0.552	1519.18
1.26	2691.94
1.68	2950.66
2.94	7123.34

Regression Output:	**PHV standard**	
Constant		0
Std Err of Y Est		510.6612
R Squared		0.962997
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	2255.538	
Std Err of Coef.	139.7196	

Batch test 1/21/95 - endogenous test #2 0.18866

solids

time	V(sample [l]	TARE WT [mg]	WT (105) [mg]	MLSS [mg/l]	WT (550) [mg]	MLVSS [mg/l]	average [mg/l]
0.00	0.01	1422.70	1436.70	1750.00	1425.50	1400.00	
0.00	0.01	1428.80	1443.00	1775.00	1431.40	1450.00	1425.00
19.00	0.01	1425.40	1437.90	1562.50	1427.30	1325.00	
19.00	0.01	1424.70	1444.50	1650.00	1428.20	1358.33	1341.67
24.00	0.01	1422.80	1442.10	1608.33	1426.10	1333.33	
24.00	0.01	1422.80	1440.90	1508.33	1426.20	1225.00	1279.17
32.00	0.01	1424.50	1445.60	1758.33	1427.90	1475.00	
32.00	0.01	1426.50	1447.10	1716.67	1429.60	1458.33	1466.67

nitrate data

time	area 1	area 2	conc 1	conc 2	average	regress		
0.00	4.8E+07	4.8E+07	50.90	50.47	50.68	49.84	1425.00	1425.00
3.00	4.7E+07	4.7E+07	49.55	49.30	49.43	49.07	1411.84	
6.00	4.6E+07	4.4E+07	48.11	46.98	47.55	48.29	1398.69	
8.00	4.6E+07	4.5E+07	48.67	47.10	47.89	47.77	1389.91	
19.00	4.2E+07	4.1E+07	44.69	43.76	44.23	44.93	1341.67	1341.67
21.00	4.4E+07	4.1E+07	45.99	43.22	44.61	44.41	1312.25	
23.25	4.1E+07	4E+07	43.37	42.11	42.74	43.83	1279.17	1279.17
26.25	4E+07	4E+07	42.04	41.88	41.96	43.05	1343.45	
28.25	4.3E+07	3.8E+07	45.56	40.67	43.11	42.54	1386.31	
32.00	4.1E+07	4E+07	43.45	42.77	43.11	41.57	1466.67	1466.67

conc	area
0.00	0.00
5.00	4381115
10.00	9640662

Regression Output: **nitrate standard**		
Constant		0
Std Err of Y Est		277785
R Squared		0.99669
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	946498	
Std Err of Coef.	24845.8	

Regression Output: **nitrate data**		
Constant		49.844
Std Err of Y Est		1.36073
R Squared		0.82599
No. of Observations		20
Degrees of Freedom		18
X Coefficient(s)	-0.25868	
Std Err of Coef.	0.02798	

PHB

time	mass 1	mass 2	area 1	area 2	% TSS	% TSS	average
0.00	47.60	51.40	3903.45	4213.95	6.07	6.06	6.07
3.00	51.80	47.90	N/A	2618.20	N/A	4.04	4.04
6.00	51.70	41.30	1775.60	3036.79	2.54	5.44	3.83
8.00	42.60	46.20	1164.98	2253.94	2.02	3.61	2.85
19.00	42.80	39.50	2350.11	2712.56	4.06	5.08	4.55
21.00	39.20	50.10	1547.76	1900.09	2.92	2.81	2.86
23.25	30.60	33.70	1759	1644.69	4.25	3.61	3.92
26.25	41.90	42.70	1840.56	2633.23	3.25	4.56	3.91
28.25	42.10	44.00	1685.61	2053.25	2.96	3.45	3.21
32.00	38.60	48.80	1296.62	1404.71	2.48	2.13	2.29

PHB standard

conc	area
0.00	0
1.67	1829.41
2.81	3506.98
6.31	9830.82
10.49	13542.5

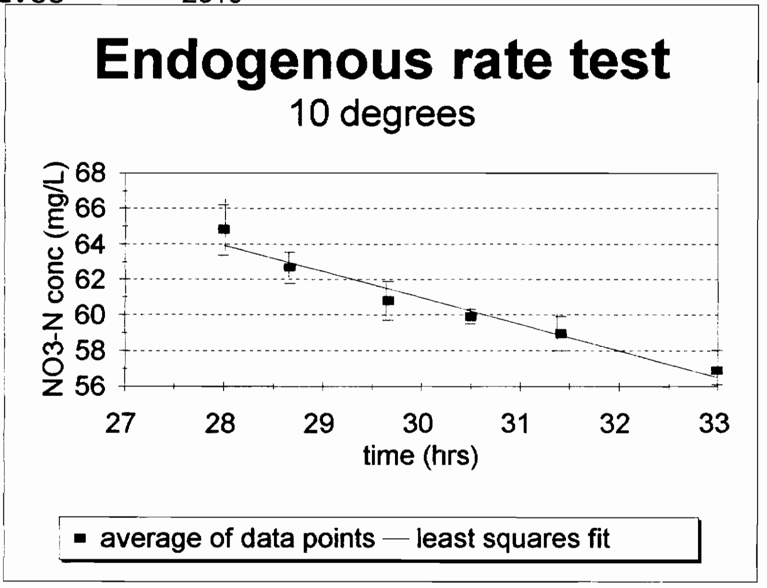
Regression Output: **PHB standard**		
Constant		0
Std Err of Y Est		770.582
R Squared		0.98185
No. of Observations		5
Degrees of Freedom		4
X Coefficient(s)	1351.89	
Std Err of Coef.	60.8264	

Endogenous rate test - endogenous test #3

run at ten degrees, MLVSS = 2610

data set 1

time	area	conc
28	5.2E+07	66.23864
28.66667	5E+07	63.5851
29.66667	4.9E+07	61.90061
30.5	4.8E+07	60.30249
31.41667	4.7E+07	59.93141
33	4.6E+07	58.01245



data set 2

time	area	conc	
28	5E+07	63.39461	
28.66667	4.9E+07	61.79426	
29.66667	4.7E+07	59.72559	
30.5	4.7E+07	59.51997	
31.41667	4.6E+07	57.98011	
33	4.4E+07	56.09233	
33	4.5E+07	56.5449	(triplicate point)

standard

conc	area
0	0
5	3812421
10	8685895
50	3.9E+07

Regression Output:	**NO3-N std**	
Constant		0
Std Err of Y Est		455581.6
R Squared		0.99936
No. of Observations		4
Degrees of Freedom		3
X Coefficient(s)	792336.2	
Std Err of Coef.	8892.041	

average values of 2 data sets

time	conc	regress
28	64.81663	63.97564
28.66667	62.68968	62.9802
29.66667	60.8131	61.48705
30.5	59.91123	60.24277
31.41667	58.95576	58.87405
33	56.88323	56.5099

regression of average values to find endogenous nitrate uptake rate:

Regression Output:	**endogenous rate**	
Constant		105.7838
Std Err of Y Est		0.612748
R Squared		0.961637
No. of Observations		6
Degrees of Freedom		4
X Coefficient(s)	-1.49315	
Std Err of Coef.	0.149117	

0.572087 mg NO3-N removed/hr/g MLVSS

Slowly Biodegradable Substrate denitrification rate test

test run at 20 degree C ambient temp, while sludge had been obtained from plant at approx. 7 degrees C

set #1

time	area	conc mg/l-N	regress 1	regress 2
0	4E+07	42.43612	42.805512	
10	4E+07	42.17736	42.400394	
20	4E+07	42.12131	41.995277	
30	4E+07	41.98719	41.590159	41.88285
40	3.9E+07	41.38744	41.185042	41.41687
50	3.9E+07	41.01307	40.779925	40.95089
65	3.8E+07	40.06196	40.172249	40.25192
80	3.8E+07	39.61994	39.564573	39.55295
95	3.7E+07	38.62194	38.956896	38.85398
110	3.6E+07	38.37291	38.34922	38.15501

Regression Output:	**all points-set #1**	
Constant		42.80551
Std Err of Y Est		0.269958
R Squared		0.971818
No. of Observations		10
Degrees of Freedom		8
X Coefficient(s)	-0.04051	mg/L/min
Std Err of Coef.	0.002439	

Regression Output:	**last 7 points set #1**	
Constant		43.28079
Std Err of Y Est		0.177489
R Squared		0.986223
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-0.0466	mg/L/min
Std Err of Coef.	0.002463	

set #2

time	area	conc mg/l-	regress 1	regress 2
0	4.1E+07	42.94491	42.988214	
10	4E+07	42.36366	42.615367	
20	4E+07	42.23505	42.24252	
30	4E+07	41.97641	41.869673	42.03908
40	4E+07	41.80807	41.496826	41.63225
50	3.9E+07	41.0712	41.123979	41.22543
65	3.8E+07	40.57731	40.564708	40.6152
80	3.8E+07	40.25278	40.005438	40.00497
95	3.7E+07	39.14346	39.446167	39.39473
110	3.7E+07	38.86693	38.886897	38.7845

set # 2

Regression Output:	**all points set #2**	
Constant		42.98821
Std Err of Y Est		0.20301
R Squared		0.981007
No. of Observations		10
Degrees of Freedom		8
X Coefficient(s)	-0.03728	mg/L/min
Std Err of Coef.	0.001834	

Regression Output:	**last 7 points set #2**	
Constant		43.25954
Std Err of Y Est		0.195657
R Squared		0.978214
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-0.04068	mg/L/min
Std Err of Coef.	0.002715	

nitrate std

time	area	regressior
0	0	0
5	4381115	4732488
10	9640662	9464976

Regression Output:	**nitrate std**	
Constant		0
Std Err of Y Est		277784.6
R Squared		0.996688
No. of Observations		3
Degrees of Freedom		2
X Coefficient(s)	946497.6	
Std Err of Coef.	24845.81	

averaged values

time	conc	regress 1	regress 2
0	42.69052	42.89686	
10	42.27051	42.50788	
20	42.17818	42.1189	
30	41.9818	41.72992	41.96096
40	41.59776	41.34093	41.52456
50	41.04214	40.95195	41.08816
65	40.31964	40.36848	40.43356
80	39.93636	39.78501	39.77896
95	38.8827	39.20153	39.12436
110	38.61992	38.61806	38.46975

Regression Output:		all points, averages
Constant		42.89686
Std Err of Y Est		0.214162
R Squared		0.980588
No. of Observations		10
Degrees of Freedom		8
X Coefficient(s)	-0.0389	
Std Err of Coef.	0.001935	

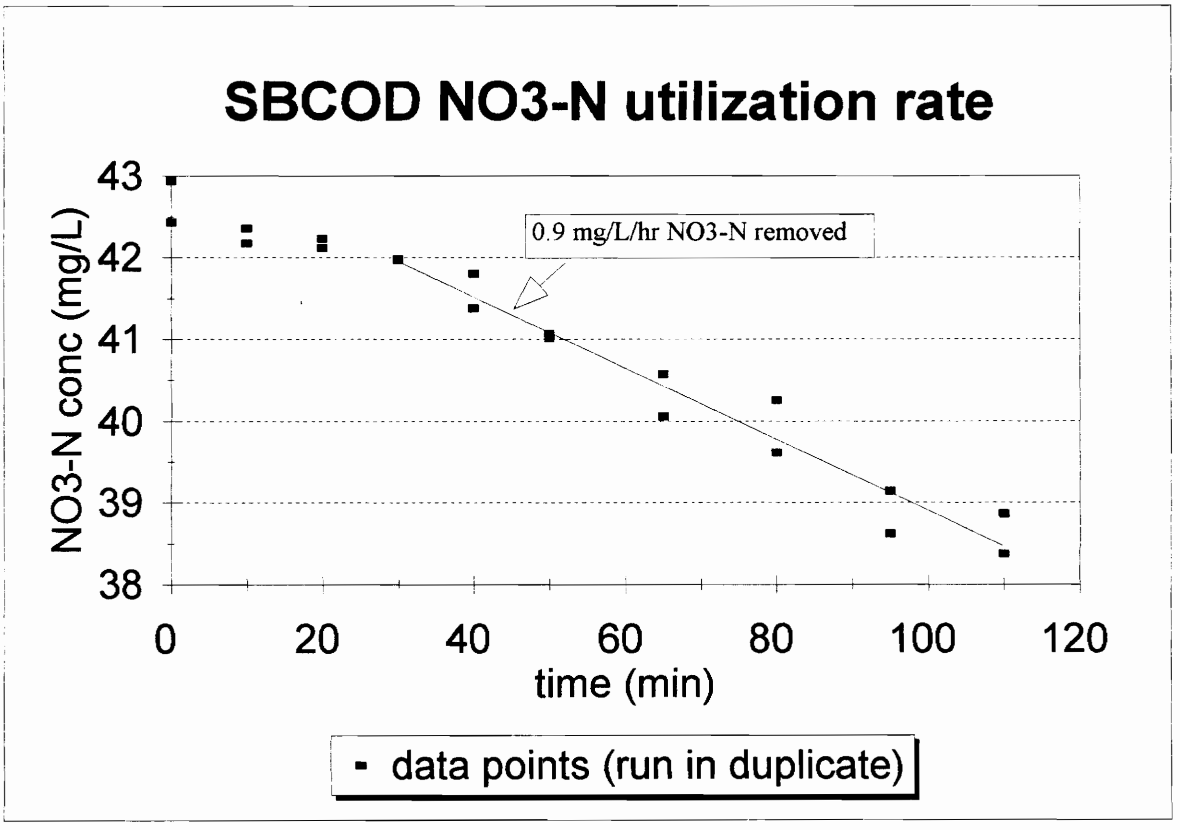
regress w/o first 3 data points:

Regression Output:		last 7 pts, averages
Constant		43.27016
Std Err of Y Est		0.159129
R Squared		0.987359
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-0.04364	
Std Err of Coef.	0.002208	

2610 mg/L

summary

all points	***0.894212***	mg/L NO3-N removed
last 7 only	***1.003221***	mg/L NO3-N removed



VITA

Patrick Brooks was born on June 6, 1971 in Bethesda, MD. He graduated from Virginia Polytechnic Institute and State University in May 1994 with a bachelor of science in Biological Systems and Agricultural Engineering.

In the fall of 1994, he enrolled in the Environmental Engineering program at Virginia Tech. He completed his Master's degree in April 1996.