RESPONSE TO OSMOTIC STRESS BY THE HALOALKALIPHILIC BACTERIUM *HALOMONAS CAMPISALIS*

By

John Aston

A thesis submitted in partial fulfillment of
the requirements for the degree of

Master of Science in Chemical Engineering

Washington State University
School of Chemical Engineering and BioEngineering

May 2006

To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of JOHN ASTON find it satisfactory and recommend that it be accepted.

_____
(Chair)

_____

_____

Acknowledgments

There are several individuals and groups that I would like to thank for their assistance and contribution towards my learning and enjoyment while at Washington State University. I would specifically like to thank:

- Dr. Brent Peyton, who has provided academic support and friendship going back several years prior to my time at WSU. It was through Dr. Peyton that I had the opportunity to first work in research and in chemical engineering.
- Center for Multiphase Environmental Research for providing financial support while at WSU.
- Interactive Graduate Education and Research Training (IGERT) for financial support as well as the opportunity to first work in research at WSU.
- Dr. Bernard Van Wie for going out of his way to assist me with several things, especially in acquiring the west waiver for my last year at WSU.
- Dr. Rajesh Sani for his support and advice, particularly in helping me with molecular biology, including pcr reactions and dna extractions.
- Diana Thorton and Jo Ann McCabe for putting up with all of my purchase orders and random questions.
- Ellen Yeates for all of her help in getting me squared away with stipends and research funding.
- Vicki Ruddick for her help in getting me squared away with stipend and student fees during the spring 2006 semester.

- Dr. Andrzej Paszczynski for his advice and use of his mass spectrometer in part of my research.
- Dr. Priya DasSarma of the University of Maryland for graciously giving me some bacteria cultures to work with.
- Dr. Doris Terry and her lab group at Purdue University for the protein expression work they performed with my samples.
- Microbial Insights for their work with lipid analysis with my samples.
- James Moberly for giving me feedback on my ideas as well as assisting me in trouble shooting with pcr and dna extraction.
- Last but not least I would like to thank my parents Earl and Barbara for their support throughout my education; my younger brother, Steven for hanging over my shoulder while I typed my thesis, and most importantly my wife, Flower for her love and patience.

RESPONSE TO OSMOTIC STRESS BY THE HALOALKALIPHILIC BACTERIUM *HALOMONAS CAMPISALIS*

Abstract

By John Aston, M.S.

Washington State University

May 2006

Chair: Bernard Van Wie

This thesis is broken down into four parts. The first part (Chapter One) provides a background into the study of halophilic organisms. Diversity, survival mechanisms, ecological significance, and applications are all discussed. Chapter Two discusses the primary focus of my research. The response of the haloalkaliphilic microorganism, *Halomonas campisalis*, to changes in environmental salinity is investigated. Three phenotypic changes are examined in detail: (1) growth kinetics, (2) compatibles solute accumulation, and (3) phospholipid fatty acid (PLFA) analysis. The study was completed in parallel with aerobic and denitrifying samples. Growth kinetics were found to be

highly dependent on media salinity. Under aerobic conditions optimal growth occurred at 20 g/L NaCl (0.5 h^{-1}). Under denitrifying conditions optimum growth occurred at 30 g/L NaCl (0.3 h^{-1}). The compatible solute ectoine was observed in the absence of salt as well as across the entire range of salinities examined, with optimum intracellular accumulation occurring at 90 g/L NaCl for both aerobic and denitrifying conditions. In much smaller amounts, glycine betaine was found at intermediate salinities, and hydroxyectoine was found at the highest salinities (175 g/L NaCl). PLFA analysis provided insights into cell stress at varying salinities. High ratios of trans monoenoic fatty acids indicated an increase in cell membrane permeability in the absence of salinity under both aerobic and denitrifying conditions. In contrast no such physiological marker was present at 175 g/L NaCl where growth kinetics were also depressed. This suggests that another cell stressor is responsible for decreased cell growth. It is possible that high salinity in the media begins to interfere with cation transfer between *H. campisalis* and the surroundings, a necessary exchange in order to balance the intracellular pH.

Chapter Three briefly discusses the effects of salinity on the growth kinetics of the extremely halophilic archaeon *Halobacterium salinarum* strain NRC-1. Again parallel observations were made under both aerobic and denitrifying conditions. An optimum growth rate of 0.047 h^{-1} at 200 g/L NaCl was seen with aerobic samples. Under denitrifying conditions the growth optima occurred at 150 g/L with a maximum specific growth rate of 0.04. The fourth chapter provides suggestions for future work.

Table of Contents

List of Tables

List of Figures

Chapter One

Introduction

1.1 - Introduction

1.1.1 – Halophiles - Halotolerant and halophilic microorganisms are by definition, capable of surviving and/or thriving in saline environments (Kushner 1978). One can distinguish between slight halophiles (optimal growth < 3% NaCl), moderate halophiles (optimal growth 3%-15%) and, extreme halophiles (optimal growth > 15%) by their optimal salinities for growth (Kushner 1978).

Because of their unique ability to thrive in the presence of salinity, they have many potential applications in biotechnology and bioremediation. These organisms, in some cases, produce organic compounds known as compatible solutes that may be used as osmotic stabilizers of biomolecules as well as entire cells. In addition, enzymes that retain activity in the presence of salinity are produced by halophilic and halotolerant microorganisms (Madern et al. 2000). The degradation and metabolism of organic pollutants under saline conditions are other applications of these extremophiles.

Many halophilic microorganisms have adapted to their harsh environments by developing unique survival tools. Some of these tools make them well suited for biotechnological purposes. The most immediate problem posed to microorganisms by salinity is osmotic pressure. Osmotic pressure is the result of water moving from a system of high water concentration to one of low water concentration. In the case of saline environments,

water would flow out of the cell, placing pressure on the cell membrane. To combat this, two separate strategies are employed by halophilic microorganisms. Halophilic archaea maintain an osmotic balance with their environment by accumulating high levels of intracellular salt, typically KCl (Oren 1999). This mechanism of osmoregulation requires special adaptations of the intracellular enzymes that have to function in the presence of salt. In contrast, halophilic bacteria, which are characterized by a much greater metabolic diversity, maintain a low intracellular salt concentration. Rather, they maintain osmotic balance across their cytoplasmic membrane by accumulating high concentrations of various organic osmotic solutes (compatible solutes). It is by balancing this osmotic pressure that these organisms are able to thrive over a wide range of salinities (Oren 1999).

1.1.2 - Diversity of Halophiles - Halophiles can be found in nature over the entire range of salt concentrations encountered in natural habitats. Since many saline habitats originated from evaporating seawater, their salinity levels are usually higher than that found in the ocean. This is evident by the dominance of sodium and chloride ions found in many such habitats. In addition, the pH is most often neutral or slightly alkaline, like that of seawater. Some changes in the ionic composition, such as precipitated minerals e.g., are common following evaporation as the system may become saturated (Oren 2002). In addition to near-neutral pH, microbial life has adapted to environments that combine high salt concentrations with high alkalinity. Such environments can be found in soda lakes across the globe, including the United States with pH values greater than 11 and salinities exceeding 30% (Sudge et al. 1998). The anaerobe *Halothermothrix orenii*

was isolated from a Tunisian salt lake with 20% salinity and temperatures up to 68 ° C (Cayol et al. 1994). Relative to the research presented here, *Halomonas campisalis* was isolated near the alkali Soap Lake in Washington State. Soap Lake is the last in a series of lakes in the region with no outlet other than evaporation. pH values of 9.8 and dissolved solids concentrations of 26 g/L have been measured in these lakes (Mormille et al. 1999).

Not only is halophilic diversity expressed at the phylogenetic level, since halophiles are found in all three domains of life (Oren 1999), but it is also expressed at the physiological level. Halophilic organisms typically use the same mechanisms to harness energy as non-halophiles do. This implies that halophiles may provide a broad source of microorganisms adapted to higher salinities, for future biotechnological uses. The field of genomics has also begun to provide insights into halophiles. The first complete genome sequence of a halophilic archaeon (*Halobacterium salinarum*) was recently completed (Ng et al. 2000). The future sequencing of halophiles will provide a valuable tool which may lead the way for extensive genetic engineering, thereby establishing an even more powerful biotechnological resource.

The domain Bacteria contains many types of halophilic and halotolerant microorganisms, spread over a large number of phylogenetic subgroups. The different branches of the Proteobacteria contain halophilic representatives, often having close relatives that are non-halophilic. Halophiles are further found among the cyanobacteria, including the *Flavobacterium-Cytophaga* branch, the spirochetes, and the actinomycetes. Within the

lineages of the gram-positive bacteria, halophiles are found both within the aerobic and anaerobic branches (Oren 2000).

Most microbial metabolic processes that occur at low salt concentrations can also be found at much higher salinities. However, some processes known to occur in low-salinity environments have not yet been observed at higher salinities. Processes that have not been observed at salt concentrations above 150 g/L NaCl are autotrophic nitrification, methanogenesis based on reduction of carbon dioxide with hydrogen as electron donor, methanogenesis from acetate, and oxidation of acetate by sulfate-reducing bacteria (Oren 1999).

These observations lend to the fact that bioenergetic constraints are related to the energetic costs of adapting to a saline environment. Processes that are observed at all salt concentrations include: photosynthesis processes, dissimilatory nitrate and sulfate reduction. Metabolic processes that provide little energy are not viable given the energy required to synthesize compatible solutes (Oren 1999).

1.1.3 – Bioremediation - The ability of halophilic and halotolerant microorganisms to oxidize hydrocarbons in high salinity can be utilized for the bioremediation of saline environments that may be contaminated with toxic hydrocarbons such as petroleum, pesticides, and PAH's. Oil rich saline wastewater is a common waste product of petroleum refinery (DoAaz et al. 2002). The bioremediation of oil spills has been observed in marine, arctic, and antarctic environments (Delille et al. 1998). Specifically,

a halo and thermotolerant, *Streptomyces albaxialis*, was found to degrade crude oil and petroleum products even in the presence of 30% NaCl (Kuznetsov et al. 1992).

Salt marshes are occasionally polluted by crude oil spills. Bioremediation may be an effective method in removing oil without damaging this ecosystem. PAH's and alkanes degraded simultaneously in microcosm laboratory studies when 0.7 g crude oil/g soil was applied (Jackson and Pardue 1999). Seasonal studies from the same site demonstrated that the mineralization of model alkanes and PAH's was uncoupled (Jackson and Pardue 1997). Low degradation rates (between 0% and 3.9% per day) of the alkane component but, high degradation rates (between 8% and 16% per day) of the PAH fraction were found.

Woolard and Irvine (1994) demonstrated the applicability of heterotrophic, halophilic bacteria for the treatment of highly saline wastewaters using a reactor. A biofilm of halophiles, isolated from the Great Salt Lake, Utah, was developed on an oxygen permeable tubing surface and could degrade more than 99% of phenol from waste brine with 15% NaCl. Hinteregger and Streichsbier (1997) studied the ability of a halophilic *Halomonas* sp. for the bioremediation of saline phenolic wastewater. This strain was capable of degrading 0.1 g phenol/L as a sole carbon and energy source in saline wastewater containing 1% to 14% NaCl with optimal growth at 5% NaCl. A microorganism capable of degrading phenol, *Alcaligenes faecalis*, was isolated from Amazon rain forest soil that had likely never been contaminated with man made phenolic compounds (Bastos et al. 2000).

Halogenated organic compounds are also an environmental concern due to their persistence and toxicity. A slightly haloalkaliphilic *Nocardioides* sp. has shown a broad range of chloro-phenol degradation, including 2,4-dichlorophenol; 2,4,5-trichlorophenol and up to 1.6 g 2,4,6-trichlorophenol/l as a sole energy source (Maltseva and Oriel 1997).

Organic solvents are another common source of environmental and industrial contamination. Aerobic transformation of formaldehyde by a moderately halophilic bacterium in the presence of between 1% and 20% salt was described by Oren et al. (1992). Mineralization of N,N, dimethylformamide by a mixed community of bacterium was documented at varying salinities. A newly discovered halotolerant microorganisms of the *Brevibacterium* genera, capable of degrading cyclohexanone and cyclohexanol in the presence of 10%-15% NaCl was recently isolated from an industrial wastewater treatment plant. Cyclohexanone oxidation is inducible and the genes of two enzymes responsible for the oxidation were identified, isolated, and expressed in *E. coli* (Brzostowicz 2000).

1.1.4 - Ecological Importance of Halophiles- Compatible solutes accumulated to high concentrations represent a significant source of carbon and nitrogen in hypersaline ecosystems. Compatible solutes may be released into the environment through passive diffusion across the cell membrane, upon cell death, or during cell lysis by bacteriophages, viruses, or parasitic bacteria (Welsh 2000). Additionally, a sudden osmotic down-shock can cause a rapid release of compatible solutes from cellular efflux

systems that somewhat mimic those of mechanosensitive channels whose probability of being activated is regulated by membrane permeability (Welsh 2000). In most environments however, changes in salinity will either be non-existent or will be gradual and occur over periods of days, weeks or months. When changes occur gradually, compatible solute concentrations within the cells are likely determined by changes in compatible solute synthesis, degradation, and cell density. Thus, the significance of rapid mechanosensitive efflux systems may be limited to particular niches which are subject to very rapid dilution. Once released to the environment, compatible solutes can serve as osmoprotectants or as carbon and nitrogen sources for other members of the community.

Most microorganisms possess transport systems for compatible solutes whose activity is directly regulated by osmotic pressure. These transport systems also serve to scavenge compatible solutes released into the environment by other microorganisms. Many bacteria possess transport systems for compatible solutes which they are unable to synthesize, allowing them to uptake solutes from the environment (Pfluger and Muller 2004). For example, many heterotrophic bacteria are unable to synthesize glycine betaine *de novo*; however they do possess a glycine betaine transport system that allows for the uptake of glycine betaine from the environment. Generally, these bacteria are also incapable of utilizing glycine betaine as a growth substrate and the expression of the transport systems are regulated by environmental salinity (Oren 1999). Thus, the only known purpose of these transport systems is likely the harvesting of environmentally available glycine betaine as a compatible solute. In some bacteria, glycine betaine transport systems may also serve to aid in the accumulation of other compatible solutes.

This was demonstrated in *E. coli* where the uptake of ectoine under osmotic stress was shown to be dependent on the *proP* and *proU* encoded glycine betaine transport systems (Jebbar et al. 2000). This dual functionality may be a common feature of glycine betaine transport systems as suggested in a recent study of natural seawater samples. In this study it was demonstrated that dimethylsulfoniopropionate (DMSP) and glycine betaine competed for the same transport systems (Diaz and Taylor 1996). Recognizing that compatible solutes are utilized as substrates in at least some bacteria, these transport systems can also function in the uptake of compatible solutes as growth substrates. In some glycine betaine and DMSP metabolizing strains, osmotic stress suppresses the metabolism of glycine betaine and DMSP. These compatible solutes then accumulate in the cytoplasm and are utilized as compatible solutes (Diaz and Taylor 1996). Microbes that possess compatible solute transport systems likely have an ecological advantage, given the low energy cost of compatible solute uptake relative to synthesis.

1.2 - Survival Mechanisms of Halophiles

1.2.1 – Cell Wall Adaptations - Prokaryotic cell membranes are comprised of both proteins and lipids. Generally, it is the lipids that adjust to environmental changes to maintain structural integrity (Prescott et al. 2003). Most lipids within the cell membrane are structurally asymmetric with polar and non-polar ends. The polar ends, comprised of hydrophilic phosphorus heads, interact with water and are typically referred to as phospholipids. The non-polar, hydrophobic ends (carbon chains) do not interact with water, but instead associate with one another. This property of lipids enables them to

form a bi-layer within the membrane. In this case, the lipids align opposite from each other with the hydrophilic ends facing out. The hydrophobic ends, comprised of the carbon chains face each other, are essentially buried within the cell membrane (Prescott et al. 2003).

The ability of bacteria to adapt to fluctuations in salinity depends on two separate adaptive responses. The first adaptation is the accumulation of solutes that are compatible with cell function. These serve to prevent water from rapidly leaving the cell, which prevents the rupturing of the cell membrane leading to cell death (Galinski and Truper 1994). The second major adaptive response to fluctuations in salinity involves alterations in the phospholipid composition of the cells cytoplasmic membrane in order to maintain the necessary hydrophilic outer layer along with the hydrophobic inner layer (Russell et al. 1985). As the salinity of the environment changes, the solubility of the phospholipid fatty acids is changed. In order to maintain the integrity of the membrane, cells change the structure of the membrane fatty acids in an attempt to maintain a constant level of membrane solubility and permeability. Such modifications of the cytoplasmic membrane composition are likely responsible for maintenance of the membrane in a stable bi-layer configuration (Sutton et al. 1991). In addition to changes in the phospholipid fatty acid structures, the fatty acid compositions of these lipids have also been observed to change. Trans and cyclic fatty acids have been shown to increase at the expense of saturated and branched straight chain fatty acids when gram-negative halophilic bacteria are exposed to increasing salinities (Russell 1989). As an example, the lipid membrane of a halotolerant *Micrococcus* strain was shown to be strongly

influenced by salinity. Increasing fatty acid chain length was seen with growth at increasing salinities. Additionally, terminally branched saturate fatty acids were seen to decrease (Nicolaus et al. 2001). Also, recent lipid analyses on the newly discovered halotolerant microorganism, *Oceanomonas baumannii* showed increases in fatty acid chain length with increasing phenol concentration (Brown et al. 2000). A general trend was also seen in this study for an increase in the ratio of saturated to unsaturated fatty acids with salinity (Brown et al. 2000).

1.2.2 - Osmotic Adaptations- Biological membranes are permeable to water; therefore, the water activity of the cytoplasm cannot be higher than that of the surrounding environment as there would be no chemical potential driving force. Because of this, high salinities may cause microorganisms' cytoplasm to be at least iso-osmotic with the outer environment. In turn, this requires a hyperosmotic cytoplasm (Oren 1999).

As discussed earlier, two separate, but similar strategies exist to cope with the osmotic stress in the presence of salinity. In one strategy, cells maintain high intracellular KCl concentrations ("salt-in strategy"); the amount of intracellular salt must be osmotically equivalent to the external conditions. In this strategy, all intracellular systems must be adapted to high salinity, limiting their functionality at low salt concentrations. A second strategy allows cells to maintain a low intracellular salinity ("compatible solute strategy"). The osmotic pressure of the medium is balanced by organic compatible solutes. In this case, the intracellular mechanisms do not necessarily need to be salt

dependent (Pfluger and Muller 2004), allowing the cells to survive over a potentially larger range of environmental salinities.

The salt-in strategy is used by two separate groups: aerobic extremely halophilic archaea of the order *Halobacteriales* (Bayley and Morton 1978) and the anaerobic halophilic bacteria of the order *Haloanaerobiales* (Oren 1986). As they rely on high levels of intracellular salts, these microorganisms do not produce compatible solutes. Most studies performed with these types of bacteria and archaea indicate that the intracellular salt levels approximate the salinity of the surrounding environment. Although environmental salinity generally consists of NaCl, the ionic composition of the cytoplasm in these microorganisms may be different. Specifically many rely on KCl to provide intracellular salinity (Oren 1999).

For microorganisms that rely on the "compatible solute strategy", osmotic balance is provided by small organic molecules that are synthesized by the cells and/or taken up from the environment. Compatible solutes are solutes which, at high concentrations, allow enzymes to function efficiently and maintain osmotic balance across the cellular membrane. There are many different types of these solutes found within halophilic and halotolerant microorganisms. Compatible solutes that have been documented include polyols, sugars and sugar derivatives, amino acids and their derivatives, as well as quaternary amines such as glycine betaine (Oren 1999). Compatible solutes are typically low molecular weight compounds that are soluble at high concentrations in water, and either uncharged or zwitterionic at the physiological pH (Oren 2002). Some compatible

solutes have been shown to be more efficient than others in protecting enzymes from the harmful effects of high salinity as well as other stresses. Intracellular concentrations of compatible solutes are regulated by the salinity of the medium, leading to a high level of adaptability to changes in environmental salinity.

Many bacteria that accumulate compatible solutes were also reported to contain high intracellular Na+ and K+ concentrations (Ventosa et al. 1998). However, since cellular proteins of *Halomonas elongata* are somewhat richer in acidic amino acids than those of non-halophilic microorganisms, it may be that some degree of salt adaptation of the intracellular machinery is required in organisms that primarily use compatible solutes for osmotic adaptation (Oren 2002). Whether microorganisms maintain intracellular salt concentrations, or synthesize compatible solutes to reduce the osmotic gradient across the cell membrane, life in the presence of high salt concentrations is energetically costly.

1.2.3 - Organic Osmotic Solutes-Energetic Aspects- Many different compatible solutes have been detected in halophilic and halotolerant microorganisms. The bioenergetic requirements for several of these compatible solutes were calculated by Garty in 1977. The study reported the results from two separate calculations, one relating to autotrophic growth and one relating to aerobic heterotrophic growth. For autotrophic microorganisms, the cost of compatible solute synthesis from CO_2 was estimated based on the mechanisms of the Calvin cycle. For heterotrophs, glucose was chosen as the substrate, and the mechanisms of the Embden-Meyerhof pathway were used. Calculations were based on the decreased production of ATP when compatible solutes

were synthesized as compared with the maximum ATP yield during complete oxidation of the equivalent amount of glucose to CO_2. Consumption and formation of one NADH or NADPH molecule was taken to be equivalent to the generation of three ATP molecules. Between 30 and 109 molecules of ATP appear to be required for the autotrophic biosynthesis of one molecule of the different compatible solutes, and for the heterotrophs between 23 and 79 potential ATP molecules that could otherwise have been generated during respiration are used per molecule of compatible solute synthesized. Specifically of interest to the research presented here, ectoine required between 50 and 60 ATP molecules under autotrophic synthesis and around 40 in the case of heterotrophic synthesis (Garty 1971).

These values may be compared to the energy required for the synthesis of cellular components in order to put them into perspective. One study estimated the ATP requirements for the synthesis of cell components (Stoutheimer 1973) suggested that 6.5 g of microbial cell material was formed from CO_2 per mole of ATP under autotrophic conditions. Obviously, the production of compatible solutes is costly from an energy standpoint. The use of smaller molecules as compatible solutes such as glycerol, glycine betaine, ectoine, and hydroxyectoine consumes less energy compared with larger compatible solutes such as trehalose and sucrose.

Typically, solutes that require less energy to produce are found in microorganisms that grow at the highest salinities. It has been shown that slightly halophilic/halotolerant microorganisms commonly use sucrose and /or trehalose as osmoprotectants, moderate

halophilic/halotolerant microorganisms often use glucosylglycerol, and the extremely halophilic/halotolerant types almost always use glycine betaine, ectoine, and hydroxyectoine (Mackay et al. 1984). From an energetic standpoint, the higher molar concentration required at high salinities, is offset by the type of compatible solute used. However, this may not be the sole reason for the use of such solutes as glycine betaine and ectoine. It has been suggested that the more energetically costly solutes, sucrose and trehalose, are not highly effective in supporting the activity of salt sensitive enzymes at high concentrations (Galinski 1993).

Another reason that lighter compatible solutes may be beneficial at higher salinities is that they help to maintain cell buoyancy. The solubility of sucrose in water is limited to about 3 M at room temperature. A 2 M solution of sucrose has a density of 1.24 g/ml, higher than the density of the most saline environments (1.235 g/ml in the Dead Sea). Therefore, cells that maintain intracellular sucrose concentrations above 2 M may be expected to sink to the bottom of salt lakes, limiting their access to important resources such as oxygen and light (Oren 2002).

The energy cost of compatible solutes is offset in many cases since they may also be used as carbon reserves. There are limitations to this idea however. In a saline environment, cells must maintain osmotic balance across the cytoplasm, and therefore will not recruit their intracellular compatible solutes for other purposes. Only when the cells encounter a decrease in salinity may compatible solutes become available as a substrate. In most habitats in which halophilic microorganisms thrive the salinity remains fairly steady, so

this is probably not a significant use of compatible solutes in a nature. Even when a rapid decrease in environmental salinity occurs, certain solutes, namely glycine betaine and ectoine, are usually excreted from the cells (Fischel and Oren 1993). However, transport systems are present in the cell membrane, allowing for the recycling of the excreted solutes at a later time at a much lower energetic cost than synthesis.

Cellular membranes are not completely impermeable to compatible solutes, and some may leak out of the cells. Some microorganisms, however, are effective in retaining these solutes. For example, the algae *Dunaliella*, which has a membrane fairly impermeable to glycerol, loses less than 5% of the total glycerol produced (Wegmann 1980). Transport systems may also serve to salvage compatible solutes that would otherwise be lost to the environment from permeable membranes. The presence of transport systems for osmotic compounds reduces the energy spent on salt adaptation when compatible solutes are available from the environment.

1.3 - Biotechnology Applications

1.3.1 - Compatible Solutes- Compatible solutes have gained interest for biotechnological applications as stabilizers of enzymes, DNA, membranes, whole cells, osmotic protectants, and stress-protective agents (Margesin and Schinner 2001). One of the most abundant compatible solutes in nature is ectoine. Ectoine is particularly common in halophilic aerobic heterotrophic bacteria (Galinski 1993).

Ectoine is used to retain and stabilize the activity of enzymes such as amylase, lipase, cellulose, and protease. It is also added to enzymatic solutions for osmotic protection in an amount of 0.05% to 50%, preferably 0.1% to 25% (Toyoda et al. 1997). Ectoine and ectoine derivatives are further patented as moisturizers in cosmetics for the care of aged, dry, and irritated skin (Motitschke et al. 2000). One of the most promising applications is the use of ectoine as a stabilizer in the polymerase chain reaction (PCR) (Sauer and Galinski 1998).

In contrast to some compatible solutes, ectoine and hydroxyectoine can only be obtained by biotechnological techniques. A process known as "bacterial milking" has been established for the production of ectoine by the extremely halophilic *H. elongata*. This strain produces compatible solutes in response to the salinity of the medium. After a high-cell-density fermentation (to obtain about 48 g/L cell dry weight), cells are concentrated using cross-flow filtration. Transfer to a low-salinity medium induces rapid release of compatible solutes to achieve osmotic equilibrium. Re-incubation in a saline medium results in the re-synthesis of these compatible solutes (Sauer and Galinski 1998).

The production of ectoine and hydroxyectoine in *H. elongata* depends both on salinity and temperature. At salinities below 15% NaCl and temperatures below 25 °C, production was limited to ectoine. The hydroxyectoine content increased with salinity and temperature. At 20% salinity and 40 °C, approximately 50% of compatible solutes produced was hydroxyectoine (Margesin and Schinner 2001). This process may not be limited to harvesting ectoine and hydroxyectoine. Microorganisms that could be used

must withstand sudden and significant changes in salinity, and should also have a broad range of salinities at which it can thrive.

Another example of production of ectoine and hydroxyectoine is by the moderately halophilic *H. elongata* strain KS3, able to grow in the presence of 0.3% to 21% NaCl and at temperatures of between 5 °C and 45 °C. This strain also accumulates ectoine and hydroxyectoine in response to saline stress. Ectoine production can be induced immediately by NaCl addition. However, hydroxyectoine was detected only at NaCl concentrations above 1.71 M (Ono et al. 1998).

Chapter Two

Response of *Halomonas campisalis* to Saline Stress: Changes in Compatible Solute Production and Membrane PLFA Composition

Abstract

Halomonas campisalis is a salt tolerant alkaliphile (optimal growth occurring at pH 9) isolated near Soap Lake, Washington. *H. campisalis* is capable of growing under both aerobic and denitrifying conditions with optimal growth occurring at 20 and 30 g/L NaCl, respectively. Monod kinetic parameters were evaluated to determine the effect of substrate concentration on growth rates. It was observed that with increasing salinity, higher substrate concentrations were required to maximize the specific growth rate. *H. campisalis* produces high levels of compatible solutes, most notably ectoine (up to 500 mM within the cytoplasm). In addition, hydroxyectoine and glycine betaine were detected. Microorganisms that produce significant quantities of compatible solutes, particularly ectoine and hydroxyectoine, are of interest in biotechnological applications. The types and amounts of compatible solutes produced by *H. campisalis* were dependent on salinity and specific growth rate, as well as the terminal electron acceptor available (O_2 versus NO_3^-). A decrease in ectoine production was observed with NO_3^- as compared to O_2 as the electron acceptor. In addition to varying accumulation of compatible solutes, changes in the phospholipid fatty acid (PLFA) composition were detected with changing salinity. An increase in trans fatty acids was observed in the absence of salinity, a cellular response to high membrane permeability. In addition, an increase in cyclic fatty acid conformation occurred in the absence of salinity and at very high salinities, indicating cell stress. Understanding the survival mechanisms of *H. campisalis* and other extremophiles will increase the breadth and effectiveness of their utilization for industrial and environmental purposes.

2.1 – Introduction

Halophilic microorganisms must negate an osmotic gradient across their membrane in order to survive under saline conditions. Halophilic and halotolerant bacteria accomplish this via the intracellular accumulation of organic compounds known as compatible solutes (Oren 1999). These solutes require no special intracellular adaptation and are capable of providing osmotic balance in a saline environment. Compatible solutes accumulate in cells either by synthesis or environmental uptake. Uptake is a more energetically favorable option; however the proper solutes or pre-cursor compounds are not always available in the environment. Transport systems, proteins, and enzymes catalyzing the uptake and *de novo* synthesis of compatible solutes are affected by changing environmental conditions (Pfluger and Muller 2004). This, in turn, defines the organism's ability to survive under a range of salinities, as well as utilize various energy sources.

H. campisalis, a gram-negative rod, was first isolated from a salt flat near Soap Lake, Washington, an alkali saline lake in Grant County, Washington (Mormile et al. 1999). Previous work with *H. campisalis* characterized the organism as a haloalkaliphile capable of growth in denitrifying conditions (Mormile et al. 1999). *H. campisalis* has been shown to be capable of degrading toxic compounds under extreme conditions; most notable is its capability to degrade phenol in the presence of high pH and salinity (Alva and Peyton 2003). *H. campisalis* has also been shown to reduce nitrous oxide in anaerobic conditions (Boltyanskaya et al. 2004). In the same study the presence of a nitrate reductase lacking molybdenum cofactor was noted. Typically, a molybdenum

cofactor is responsible for the expression of genes that code for nitrate reductase. It is possible that *H. campisalis* employs a similar compound, such as tungsten (Boltyanskaya et al. 2004).

To date, no studies have reported phenotypic adaptations of *H. campisalis* to changing environmental salinity. Additionally, relatively little has been reported on adaptations of halophiles in general under denitrifying conditions. With many possibilities for the application of halophiles and alkaliphiles for industrial and environmental purpose, such a gap in general knowledge may hinder the full application potential of these microorganisms.

2.2 – Materials & Methods

Growth kinetics - *H. campisalis* (ATCC # 700597) was grown on a basal mineral medium containing (in g/L) KH_2PO_4, 0.5; NH_4Cl, 1.0; $Na_2B_4O_7$, 4.0; $FeCl_3$, 0.0125; $CaCl_2$, 0.06; and $MgCl_2$, 0.05. The medium was supplemented with a 1 mL/L of a trace element solution that would provide a total medium concentration of the following concentration (in mg/L) NaNO3, 10; Na_2SO_4, 10; $CoCl_2*H_2O$, 0.12; $MnCl_2*4H_2O$, 0.10; $ZnCl_2$, 0.07; $NiCl_2*6H_2O$, 0.025; $Na_2MoO_4*2H_2O$, 0.025; $CuCl_2*2H_2O$, 0.15; $MgCl_2$, 60; $CaCl_2$, 50. A varying concentration of NaCl (0-260 g/L) was also added. NaCl, KH_2PO_4, NH_4Cl, and $Na_2B_4O_7$ were added prior to autoclaving; while filter sterilized (0.2 μm) $FeCl_3$ and the trace mineral solution were added after the medium was autoclaved and cooled to room temperature. The pH was adjusted to 9.0 using 10N NaOH. Prior to

inoculation (in g/L) glucose, 1.0; $NaNO_3$ (denitrifying), 0.5; and yeast extract 0.01; were added. To provide an inoculum, glycerol preserved samples (-80°C) of *H. campisalis*, previously grown in similar conditions, were used to initiate each experiment. Culture purity was verified via 16S rDNA analysis. Aliquots of 6% (v/v) were transferred into fresh medium after the exponential growth had ceased. During late log-growth phase of the fourth transfer, samples were taken to measure cell growth. Cultures were grown in triplicate 250-mL Erlenmeyer flasks (100 ml media volume) fitted with foam stoppers for the aerobic samples. 150-mL anaerobic serum bottles with butyl rubber seals were used for the denitrifying experiments. The denitrifying cultures were purged with filtered (0.2 μm) ultra-pure N_2 for 30 minutes to ensure an anoxic environment. Both the aerobic and denitrifying samples were placed on shakers at 120 rpm in an environmental chamber maintained at 37 °C. Experiments were stopped after several consecutive measurements indicated that growth had ceased. Optical density measurements were taken at 595nm (Milton Roy Spectronic Genesys 8453, St. Petersburg, FL). Un-inoculated medium was used to calibrate the spectrophotometer.

For experiments used to determine Monod kinetic parameters, a similar protocol was followed. Cultures were grown under aerobic conditions at 30, 50, 70, and 90 g/L NaCl. At each salinity, six substrate concentrations were tested; 0.1, 0.25, 1.0, 2.0, 5.0, and 10.0 g/L glucose. The Monod Equation: $\mu = \frac{\mu_{\max} C_s}{C_s + K_s}$ was used where μ represents the specific growth rate at a given substrate concentration; μ_{max} represents the maximum specific growth rate observed with increasing substrate at a given salinity; C_s represents the substrate concentration, and K_s is the substrate concentration at which one half of μ

$_{max}$ is observed. A K_s value was found using a Microsoft excel solver package. Experiments were performed in triplicate with individual K_s values determined for each sample. The resulting Monod parameters were used to calculate specific growth rates which were compared with measured values to determine the significance of substrate concentration in the growth of *H. campisalis* with increasing salinity.

Compatible Solute Extraction and Analysis - Prior to extraction of organic solutes, 50 mL of media was inoculated with 6% (v/v) and grown for four generations in identical conditions as those described above. Cells were grown in triplicate under both aerobic and denitrifying conditions in 0, 20, 30, 90, and 175 g/L NaCl. During the late exponential phase of the fourth generation, cultures were centrifuged for 30 minutes at 7,000 rpm. Following cell centrifugation, cell material was collected and dried at 40 °C for 48 hours. Cell material was suspended in an extraction mixture consisting of 55% methanol, 25% chloroform, and 20% water. Cell material was 5% of the total mixture by weight. Following 24 hour incubation on a shaker at 120 RPM, cellular debris was removed by centrifugation for 15 minutes at 5,000 rpm. An equal volume of a separate mixture containing 50% chloroform and 50% water was added to the remaining supernatant. Following mixing by repeated inversion, a phase separation occurred. After another centrifugation at 5,000 rpm for 15 minutes, the lighter phase was recovered. An equal volume of 0.5 N perchloric acid was added at 4 °C to facilitate protein precipitation. The mixture was again centrifuged at 5,000 rpm for 15 minutes and supernatant was retained for compatible solute analysis. Electrospray-ionization Mass Spectrometry (ES/MS) (Quattro II, Micromass, Ltd., U.K.) was used to identify

compatible solutes in the final extraction product with the assistance of Dr. Andrzej Paszczynski at the University of Idaho. Solutions were delivered into the MS at a flow rate of 5 μl/min using a syringe pump (Harvard Apparatus). A potential of 3.1 kV was applied to the electrospray needle. Both protonated and de-protonated (anion and cation) conditions were used to identify compatible solutes. With the use of standards (Sigma-Aldrich), the identified compatible solutes: ectoine, hydroxyectoine, and glycine betaine were then quantified. To calculate total compatible solute production, the moles of compatible solute observed in the extraction product was converted to a molarity based on the original media volume. To calculate intracellular accumulation the dry weight of the cells used in the extraction was used to determine an overall number of cells after having calculated a cell density. The cell density was found by counting cells from a culture that contained a known cell mass. The approximate cellular volume of *H. campisalis* (~ 7.8 $X10^{-16}$ L) was then used to calculate the cytoplasmic molarity.

To test the effect of the availability of environmental compatible solutes, cultures were prepared as above, with the addition of 1 g/L of either ectoine or glycine betaine. The purpose being to determine if any change in the maximum specific growth was due to additional substrate utilization, or solely to solute uptake. Additionally, the substrate (glucose) was limited to 0.5 g/L. Experiments were performed at two different salinities, 20 and 60 g/L NaCl. In each case three conditions were examined: 1) a control in which glucose provided the sole substrate; 2) either ectoine or glycine betaine was supplied as the only substrate, and 3) glucose and either ectoine or glycine betaine were supplied. All experiments testing the utilization of environmental compatible solutes were

performed in triplicate under denitrifying conditions to limit energy availability, increasing the benefit of scavenging environmentally available compatible solutes.

PLFA analysis - Ten cultures of *H. campisalis* were grown at five different salinities: 0, 20, 30, 90, and 175 g/L and analyzed for PLFA compositions. Half of the cultures were grown in denitrifying conditions and were harvested during the exponential growth phase of their fourth generation Aerobic cultures were grown in parallel. The samples for PLFA analysis were sent overnight on ice to Microbial Insights (Rockford, Tennessee) in individual 150 ml serum bottles containing 50 mL of culture. At Microbial Insights lipids were quantified by gas chromatography and mass spectrometry, after the different lipid classes had been separated and extracted.

Statistical Analysis - Statistical significance was insured in all growth rate studies by performing all experiments in triplicate. A growth rate was determined for each sample, and the average of the three samples was assigned a standard deviation based on the variations between the three samples. An identical procedure was followed for all compatible solute data. It was not possible to collect replicate data from the PLFA analysis. Methods used by Microbial Insights yield repeatable results with a variance of less than 5% (Personal Communication Greg Davis, 2006). The error bars displayed on plots represents the standard deviation of the sample.

2.3 – Results

Growth kinetics -

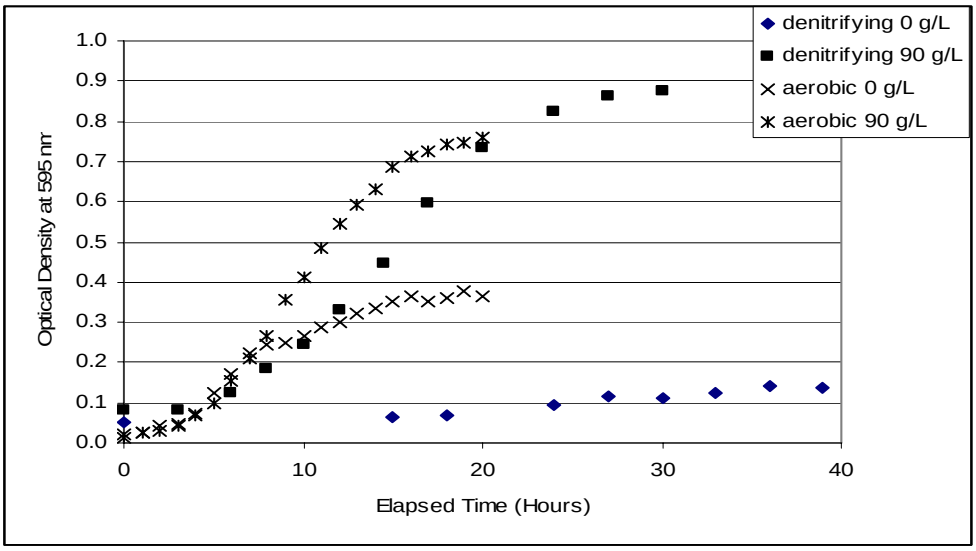


Figure 1. Growth of *H. campisalis* under aerobic and denitrifying conditions at 0 and 90 g/L NaCl.

Under aerobic conditions, *H. campisalis* grew at a wide range of salinities. From no salt conditions (maximum specific growth rate of 0.3479 h^{-1} +/- 0.033 h^{-1}) to a salt concentration of 205 g/L NaCl (maximum specific growth rate of 0.0097 h^{-1} +/- 0.0025 h^{-1}). The highest maximum specific growth rate of 0.5319 h^{-1} +/- 0.044 h^{-1} was observed under aerobic conditions at a salinity of 20 g/L (Figure 2) suggesting that *H. campisalis* is slightly halophilic under aerobic conditions. Higher variability in specific growth rate was observed at lower and intermediate salinities with standard deviations for triplicate samples ranging from 10% to 20%. At salinities greater than 175 g/L, sample to sample variability was noticeably reduced as standard deviation ranged from 0%-10%.

Under denitrifying conditions, *H. campisalis* utilized nitrate, and grew over a wide range of salinities (Figure 1). As under aerobic conditions, growth occurred between 0 and 205 g/L NaCl. A maximum specific growth rate of 0.046 h^{-1} +/- 0.004 h^{-1} was observed in the absence of salinity, while at 205 g/L NaCl, a maximum specific growth rate of 0.0074 h^{-1} +/- 0.002 h^{-1} was observed. Optimal growth under denitrifying conditions occurred at 30 g/L NaCl with a maximum specific growth rate of 0.3321 h^{-1} +/- 0.022 h^{-1}, indicating that *H. campisalis* is moderately halophilic under denitrifying conditions (Figure 2). Generally, the greatest variability (5-20%) occurred at intermediate salinities where the maximum specific growth rates were the highest (30 -100 g/L NaCl). At the lower and higher salinities, variability was below 5%.

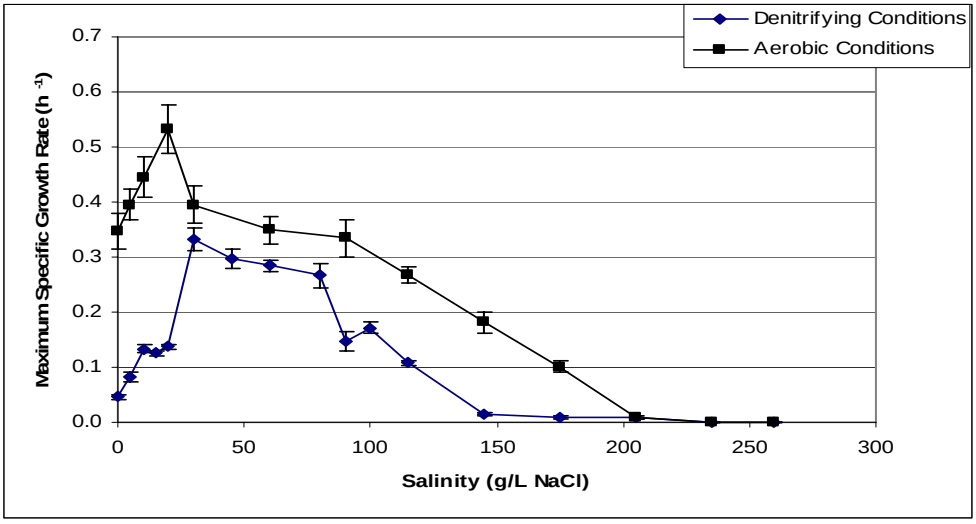


Figure 2. Dependence of *H. campisalis* maximum specific growth rate on salinity under both aerobic and denitrifying conditions.

Growth rates for *H. campisalis* increased significantly with an increase in substrate concentration. Diminished returns were observed above a substrate concentration of 2

g/L glucose (See Figure 3-6). The maximum specific growth rate observed with increasing substrate (μ_{max}), decreased with increasing salinity, while the K_s values increased with higher salinity. Monod parameters are listed in Table 1 below.

Table 1. Values of Monod parameters observed (μ_{max}) and calculated K_s.

Salinity (g/L)	μ_{max} (h^{-1})	Ks (g/L)	Ks Standard Deviation (g/L)
30	0.487	0.254	0.019
50	0.464	0.275	0.042
70	0.440	0.342	0.033
90	0.433	0.367	0.046

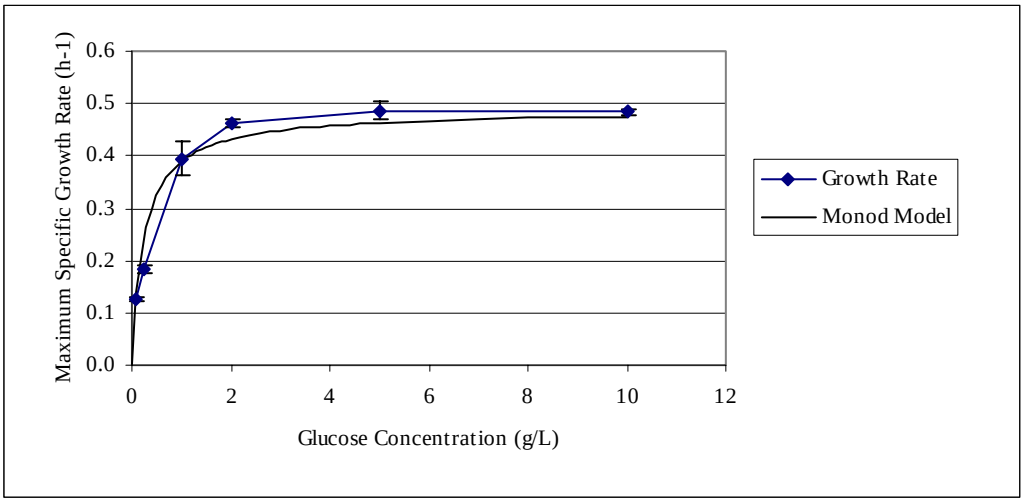


Figure 3. Maximum specific growth rate with varying substrate concentrations at 30 g/L NaCl. Observed growth rate compared to growth rate predicted by Monod model.

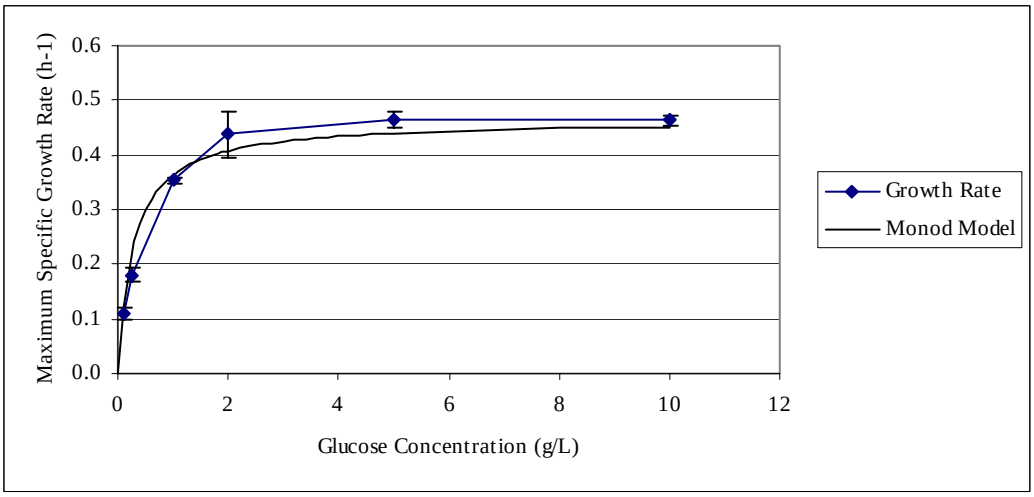


Figure 4. Maximum specific growth rate with varying substrate concentrations at 50 g/L NaCl. Observed growth rate compared to growth rate predicted by Monod model.

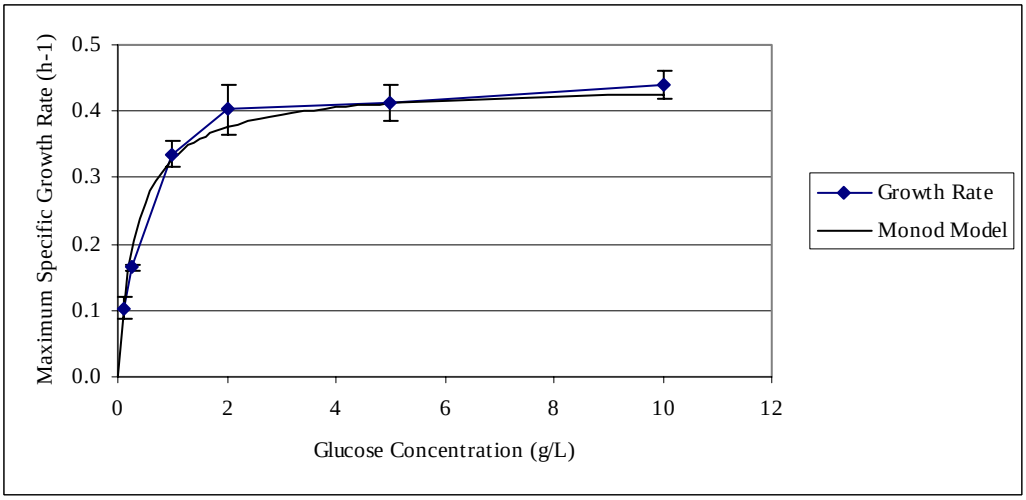


Figure 5. Maximum specific growth rate with varying substrate concentrations at 70 g/L NaCl. Observed growth rate compared to growth rate predicted by Monod model.

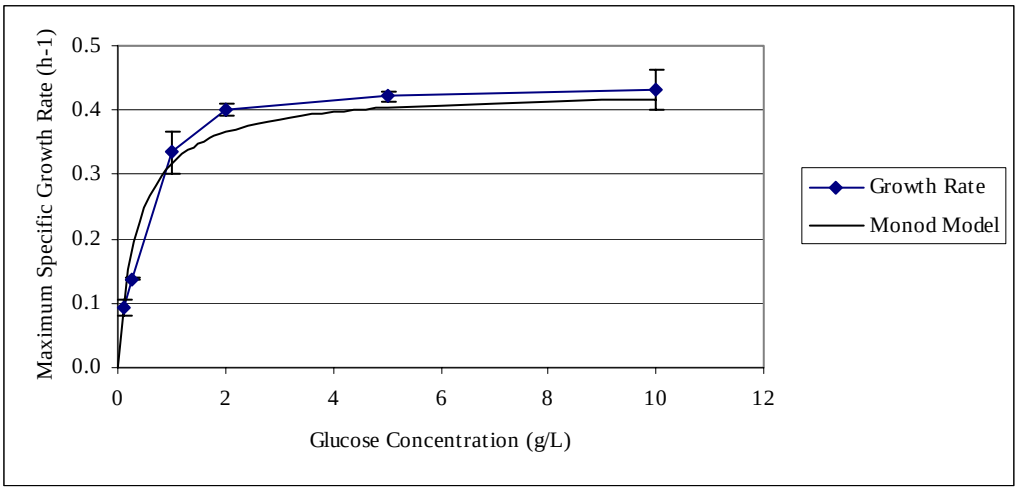


Figure 6. Maximum specific growth rate with varying substrate concentrations at 90 g/L NaCl. Observed growth rate compared to growth rate predicted by Monod model.

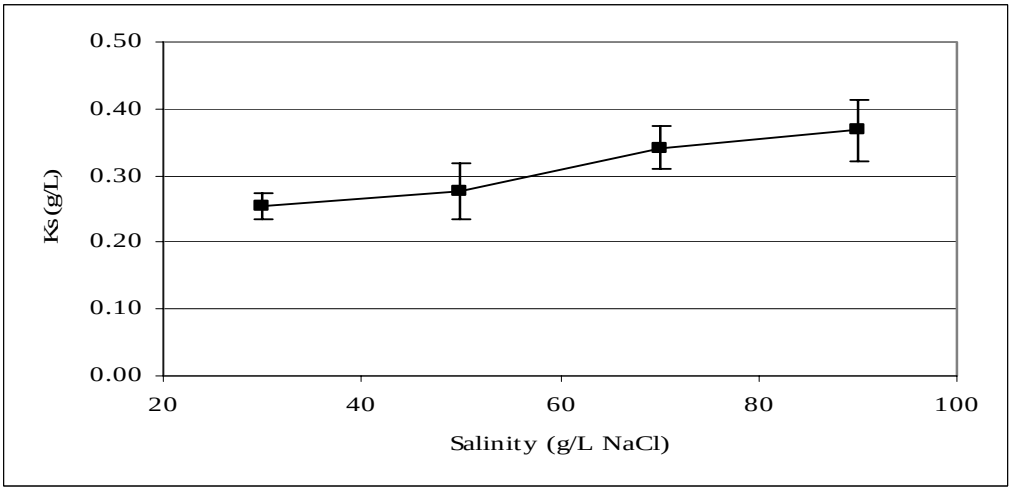


Figure 7. A plot of K_s, the substrate concentration required to achieve one half of μ_{max}, with increasing salinity.

Compatible solute analysis - Calculating concentrations of compatible solutes in the medium is of practical use for industrial applications. The general behavior of medium solute concentrations from varying salinity is the same as intracellular solute concentrations under aerobic conditions (Figure 8); the total compatible solute concentration is however, much lower, no greater than 0.15 mM. As can be seen in Figure 8, the medium solute concentration profile is relatively flat when compared to cytoplasmic solute accumulation (Figure 10) between and 90 g/L NaCl due to lower cell concentrations at the upper salinities. Under denitrifying conditions, medium solute concentrations are significantly different than that of intracellular concentrations (Figure 9). As observed in aerobic conditions, the concentration is constant from 0 to 20 g/L NaCl, and is also nearly constant at about 0.05 mM between 30 and 90 g/L NaCl.

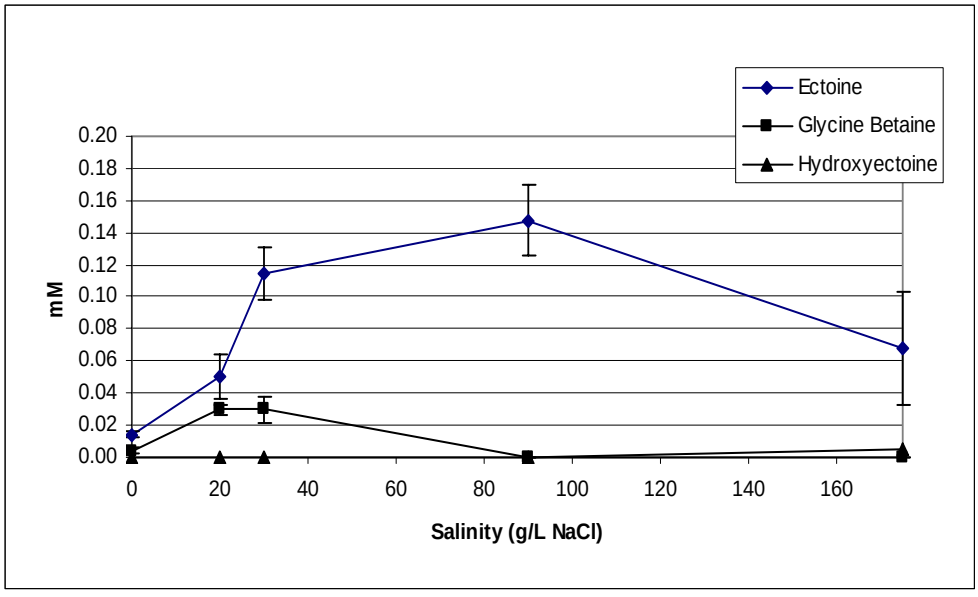


Figure 8. Production of the compatible solutes ectoine, hydroxyectoine, and glycine betaine by *H. campisalis* under aerobic conditions and varying salinities. Molarities represent concentrations based on the entire growth medium.

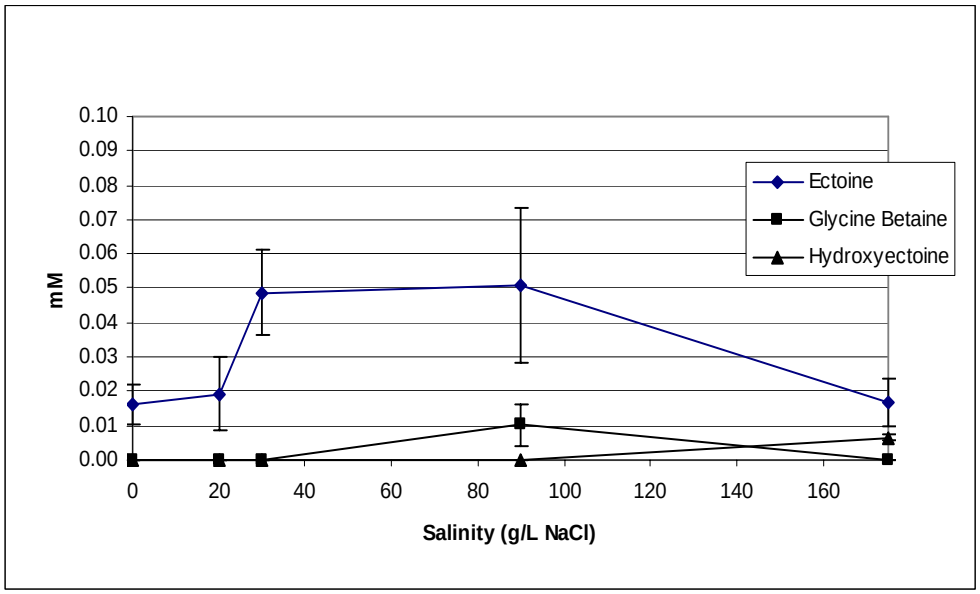


Figure 9. Production of the compatible solutes ectoine, hydroxyectoine, and glycine betaine by *H. campisalis* under denitrifying conditions and varying salinities. Molarities represent concentrations based on the entire growth medium.

It was observed that both the type and quantity of compatible solutes accumulated intracellularly by *H. campisalis* was affected by salinity, and also was dependent upon electron acceptor (O_2 versus NO_3^-) used for growth. Ectoine was the dominant compatible solute observed under all conditions, being found across all ranges of medium salinity under both aerobic and denitrifying conditions (Figure 10 and 11). Glycine betaine was detected at intermediate salinities, although the conditions at which it was found varied from aerobic (0 -30 g/L NaCl) to denitrifying conditions (90 g/L NaCl). In addition, hydroxyectoine was detected in smaller amounts at the highest salinities. Cytoplasmic solute molarity varied linearly under aerobic conditions from approximately 100 mM in the absence of salt to over 500 mM at 90 g/L NaCl (Figure 10). Cytoplasmic

compatible solute concentrations were found to be lower at 175 g/L NaCl where cell growth was limited. Under denitrifying conditions, cytoplasmic solute concentration was fairly constant near 50 mM between 0 and 20 g/L NaCl, then increased fairly linearly to 90 g/L NaCl were it approached 300 mM, before again decreasing at 175 g/L NaCl to below 100 mM (Figure 11).

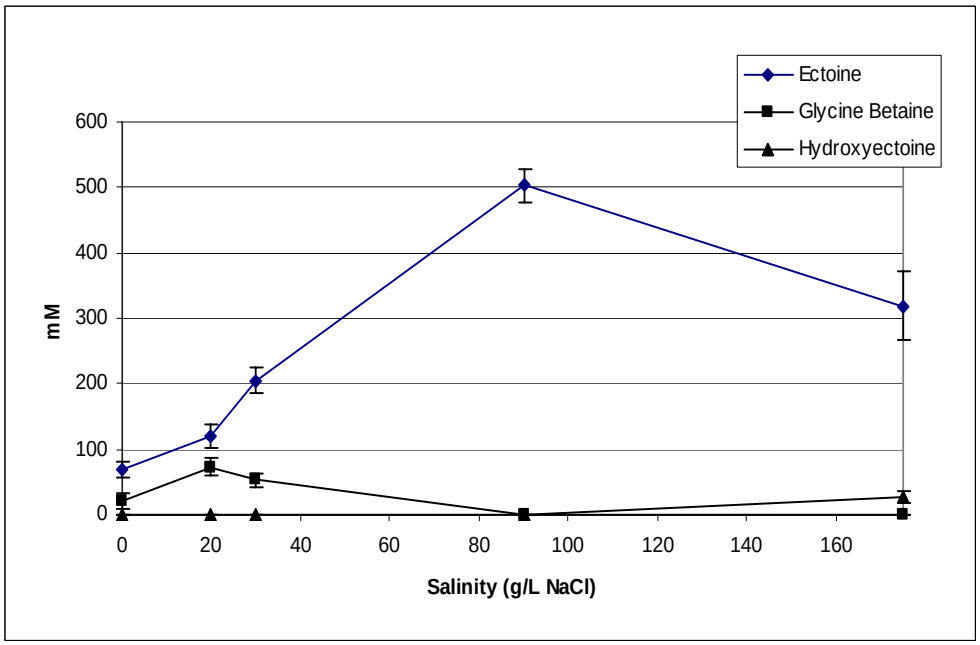


Figure 10. Cytoplasmic molarity of the compatible solutes ectoine, hydroxyectoine, and glycine betaine found in *H. campisalis* under aerobic conditions and varying salinities.

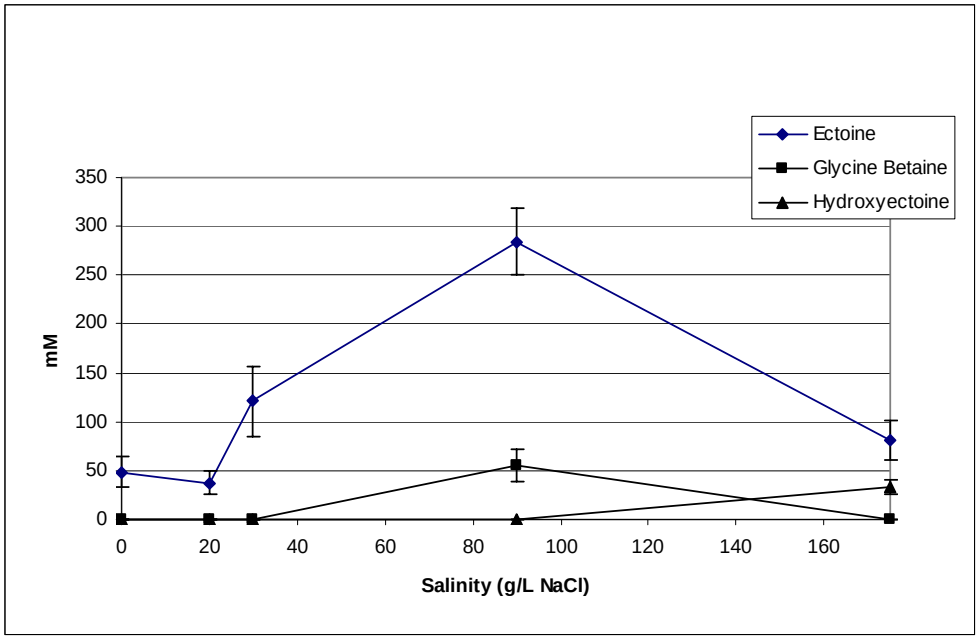


Figure 11. Cytoplasmic molarity of the compatible solutes ectoine, hydroxyectoine, and glycine betaine found in *H. campisalis* under denitrifying conditions and varying salinities.

To test the effect of environmental compatible solutes on the growth and survival of *H. campisalis* under varying salinity, the growth medium was spiked with either (1) ectoine or (2) glycine betaine under aerobic conditions. As can be seen in Figure 12, *H. campisalis* showed no significant growth when ectoine was the lone substrate. Similarly, no significant growth was observed using glycine betaine as the sole substrate (Figure 13). No benefit was observed by spiking the growth medium with 1 g/L ectoine in addition to the 0.5 g/L of glucose present in the growth medium at both 20 and 60 g/L NaCl. However, a benefit (20% increase in maximum specific growth rate) was apparent by spiking the medium with 1 g/L glycine betaine in addition to the 0.5 g/L of glucose at 60 g/L NaCl. The significance of this increased growth rate is likely statistically

significant as the 95% confidence interval for this data is +/- 0.035 h^{-1}. The difference in this case is 0.055 h^{-1}. No significant benefit was apparent at the lower salinity (20 g/L NaCl).

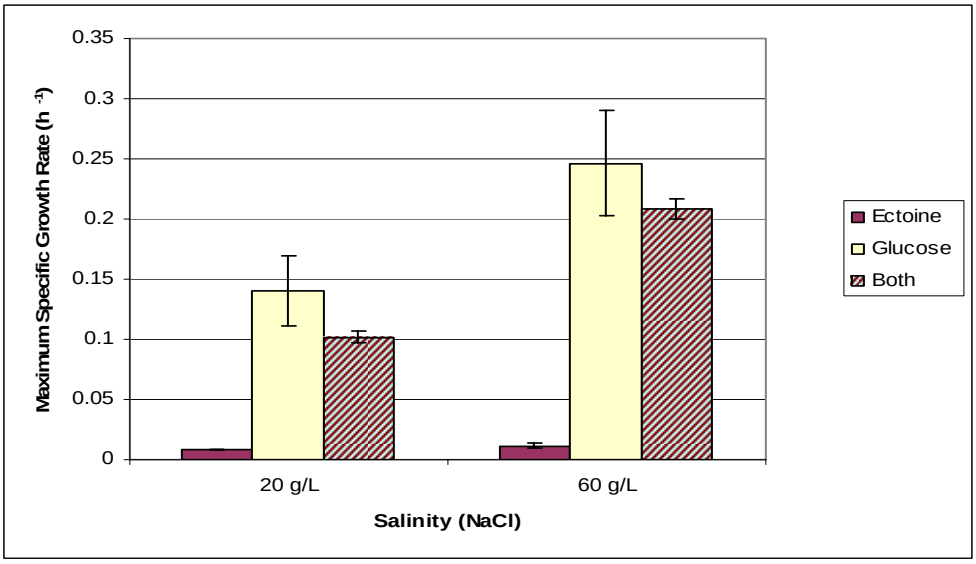


Figure 12. Effect of addition of 1 g/L ectoine on the growth kinetics of *H. campisalis* at 20 and 60 g/L NaCl.

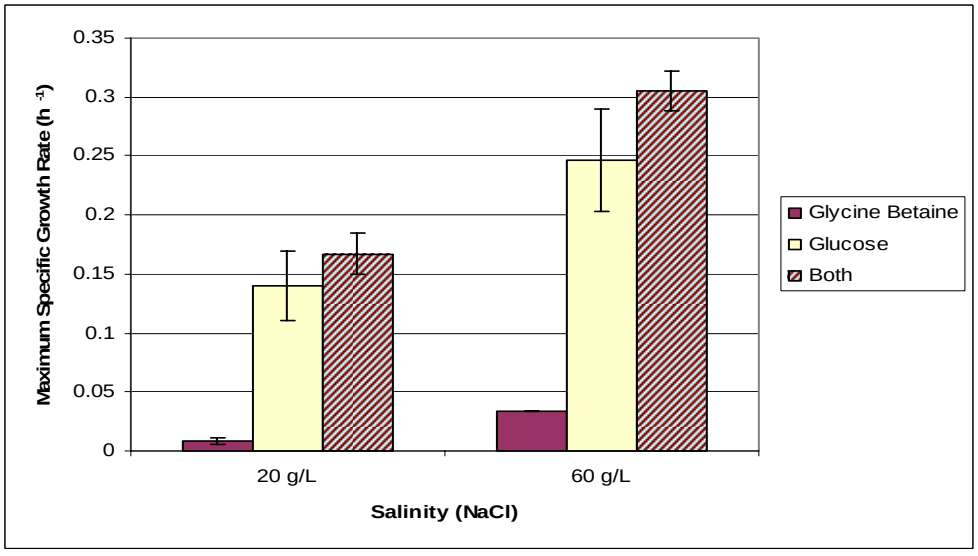


Figure 13. Effect of addition of 1 g/L of glycine betaine on the growth kinetics of *H. campisalis* at 20 and 60 g/L NaCl.

PLFA analysis - Three types of lipids were detected with PLFA analysis: Monoenoic, normal saturates, and terminally branched saturates (TBS). Monoenoic fatty acids differ from the saturated fatty acids in that they possess a single double bond. TBS fatty acids differ from normal saturates in that a methyl group will extend from an otherwise saturated fatty acyl chain. All three were present under both aerobic (Table 2) and denitrifying (Table 3) conditions, and exhibited similar changes with salinity.

Table 2. Fatty acid type percent composition with varying salinity under aerobic conditions. Methods used typically exhibit standard deviations below 5 percent.

	Salinity (g/L NaCl)				
Fatty Acid Type	**0**	**20**	**30**	**90**	**175**
Terminally Branched Saturates	8.2	0.5	0.0	0.3	0.1
Monoenoics	57.5	80.0	84.9	78.5	81.6
Normal Saturates	34.2	19.4	14.9	20.9	18.3

Table 3. Fatty acid type percent composition with varying salinity under denitrifying conditions. Methods used typically exhibit standard deviations below 5 percent.

	Salinity (g/L NaCl)				
Fatty Acid Type	**0**	**20**	**30**	**90**	**175**
Terminally Branched Saturates	20.5	0.1	0.4	0.0	0.1
Monoenoics	57.6	81.6	77.7	85.6	78.5
Normal Saturates	21.9	18.3	21.9	14.4	21.4

Under both aerobic and denitrifying conditions, terminally branched saturates were present only in the absence of salinity. Under aerobic conditions, terminally branched saturates comprised roughly 8% of the lipid composition and roughly 20% under

denitrifying conditions. Normal saturates remained relatively constant under aerobic conditions, comprising between 20% and 40% of the lipid composition. Normal saturates were nearly constant under denitrifying conditions, comprising 20% of the total lipid composition. Monoenoic fatty acids were the predominant lipid type observed under both aerobic and denitrifying conditions. Under aerobic conditions the monoenoic fatty acid composition increased from 60% (in the absence of salinity) to 80% (in the presence of salinity). In denitrifying conditions, the composition of monoenoic fatty acids was similar to those observed under aerobic conditions and increased from 60% (no salinity) to 80% (presence of salinity). There were some slight variations, but increasing salinity did not seem to have a significant impact on the composition of either monoenoic fatty acids or normal saturates fatty acids under both aerobic and denitrifying conditions.

Changes in carbon chain length of the various fatty acids were also monitored with PLFA. Terminally branched saturates appeared mostly in chain lengths of 15 and 17 carbons under aerobic conditions. Under denitrifying conditions, chain lengths varied between 15 and 17 carbon lengths in terminally branched saturates. The average chain length for the monoenoic fatty acids was 18 carbons under aerobic and denitrifying conditions. In the absence of salinity, a significant portion of monoenoic acids were found in cyclic formation in both aerobic and denitrifying conditions. Cyclic formations were seen at both 17 and 19 carbon lengths. At 30 g/L NaCl, predominantly cis formations were present in monoenoic acid, while trans formations were more prevalent at other salinities. Additionally, straight chain monoenoic fatty acids varied in chain length between 16 and 18 carbon lengths. Normal saturates behaved similarly under

aerobic and denitrifying conditions with the vast majority of fatty acid chains found to be 16 carbons in length. Noticeably, under both aerobic and denitrifying conditions, chain length appeared to depend on salinity (Tables 4 and 5).

Table 4. Overall percent compositions of fatty acid chain lengths produced under aerobic conditions. Methods used typically exhibit standard deviations below 5 percent.

	Salinity (g/L NaCl)				
Total Chain Length	**0**	**20**	**30**	**90**	**175**
14	1.2	0.2	0.7	0.1	0.3
15	4.1	0.3	0.6	0.0	0.2
16	44.7	32.5	31.3	16.3	29.6
17	18.2	32.8	9.5	6.2	2.5
18	9.9	30.7	54.4	60.3	61.7
19	21.9	3.5	3.5	17.1	5.8

Table 5. Overall percent compositions of fatty acid chain lengths produced under denitrifying conditions. Methods used typically exhibit standard deviations below 5 percent.

	Salinity (g/L NaCl)				
Total Chain Length	**0**	**20**	**30**	**90**	**175**
14	5.5	0.2	0.4	0.0	0.0
15	15.1	0.0	0.2	0.0	0.1
16	25.4	23.7	26.1	16.3	28.7
17	2.8	0.6	0.9	0.0	0.7
18	16.7	73.7	71.2	81.4	68.1
19	34.6	1.7	1.3	2.3	2.5

Of particular interest in the PLFA analysis is the observation of important physiological markers. Most notable, the ratio of trans and cis fatty acids, as well as cyclic and cis fatty

acids. In the absence of salinity, a trans/cis ratio of 1.51 was observed in aerobic samples (Figure 14) as compared to 2.41 under denitrifying conditions (Figure 15). In the presence of salinity, there was no significant production of trans monoenoic fatty acids with either O_2 or NO^-_3 as the electron acceptor. Under aerobic conditions, there was significant formation of cyclic fatty acids in the absence of salinity, as well as at the higher salinities. Cyclic to cis ratios of 3.3, 0.1, 0.06, 0.43, and 0.36 were observed at 0, 20, 30, 90, and 175 g/L NaCl (Figure 14). Under denitrifying conditions, there were few cyclic formations in the presence of salt, however a fairly large ratio of cyclic to cis fatty acids of 4.21 was observed in the absence of salinity (Figure 15).

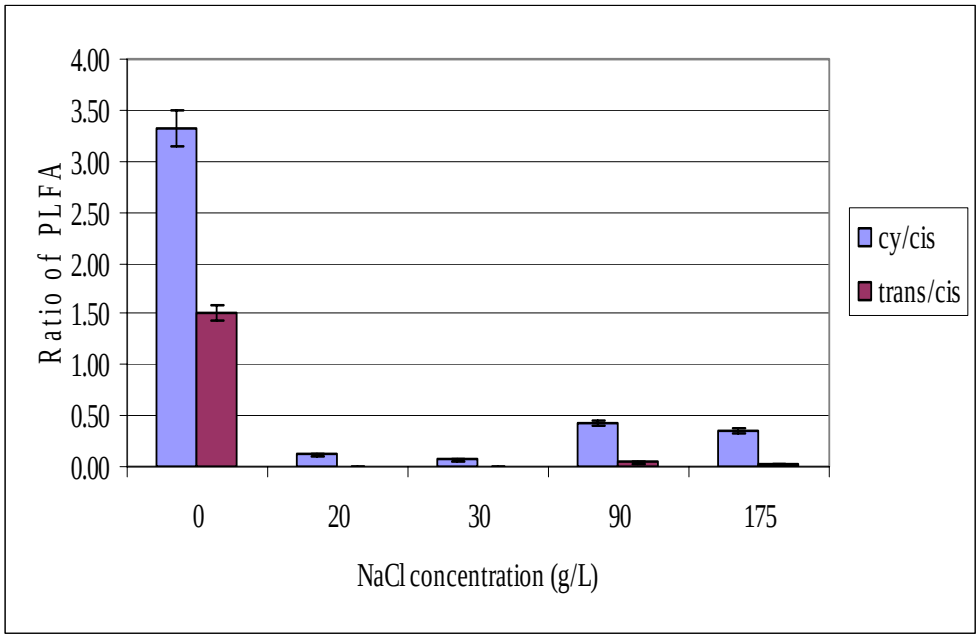


Figure 14. Physiological markers observed under aerobic conditions including the ratio of cyclic and trans fatty acids to cis fatty acids found within the phospholipid fatty acids of the cell membrane.

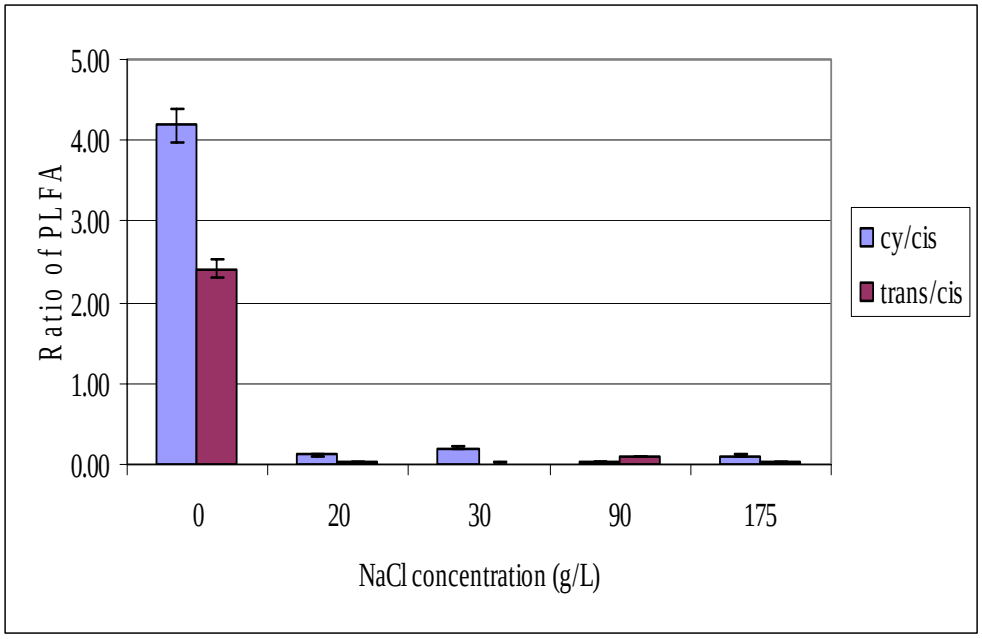


Figure 15. Physiological markers observed under denitrifying conditions including the ratio of cyclic and trans fatty acids to cis fatty acids found within the phospholipid fatty acids of the cell membrane.

2.4 - Discussion

Under both aerobic and denitrifying conditions, *H. campisalis* was able to grow in NaCl concentrations from 0 to 205 g/L. Under aerobic conditions, the maximum growth rate occurred at 20 g/L NaCl, while under denitrifying conditions, optimum growth occurred at a NaCl concentration of 30 g/L NaCl (Figure 2). Interestingly, there is a slight up-shift in optimal growth salinity when changing from aerobic to denitrifying conditions. Also of note, the upper limit of salinity in which cell growth was observed did not change from aerobic to denitrifying conditions. It would seem that as the cell has reduced access to

energy under denitrifying conditions (~0.85 Volts/mole substrate versus ~1.25 Volts/mole substrate (Prescott et al. 2003)) its upper limit for salt tolerance would decrease. The conditions most likely to challenge *H. campisalis* in a saline environment are the osmotic gradients that result from the presence of high salinity, and the absence of salinity. In this investigation, two primary coping mechanisms were observed for changes under varying salinity: compatible solute production and modification of phospholipid fatty acid composition. In studying the growth kinetics of *H. campisalis* along with patterns in compatible solute accumulation and lipid composition adaptations, insights were gained into the survival mechanisms and adaptations of this halophilic microorganism.

Compatible Solute Analysis - Examining the variations of compatible solutes produced by *H. campisalis* under varying salinities gives information that is important in understanding the full picture of how *H. campisalis* adapts to salinity; as well as how this may be used with this or other halophiles for industrial purposes. Under anoxic conditions, ectoine is the dominant compatible solute produced in the absence of salinity, which accumulated intracellularly to 48 mM +/- 16 mM. Interestingly, with a slight increase in salinity to 20 g/L, intracellular ectoine accumulation remained statistically the same at below 50mM. At low salinities, 20 and 30 g/L NaCl, ectoine accumulation was measured to be 38 mM +/- 12 mM and 120 mM +/- 36 mM, respectively. However, over this same change in salinity, the maximum specific growth rate nearly tripled from 0.046 h^{-1} to 0.1374 h^{-1}. This may be due to the osmotic balance provided by the environmental salinity. Maximum ectoine accumulation was observed at 90 g/L (284 mM +/- 34 mM).

At this salinity, glycine betaine was also detected in significant amounts (55 mM +/- 10 mM). The growth kinetics are not maximal at this salinity suggesting that either, osmotic balance is not optimized, or salinity is interfering with cell growth through some other mechanism. Brown et al. (2002) discuss the possibility of internal compatible solute accumulation becoming toxic to a cell at high concentrations. Perhaps this is the reason for declined growth rates at 90 g/L NaCl, given the robust compatible solute accumulation. At the highest salinity where compatible solutes were measured, 175 g/L NaCl, ectoine concentrations were found to be 82 mM +/- 20 mM. In addition, hydroxyectoine was detected at a concentration of 27 mM +/- 8 mM (Figure 9).

Compatible solute production under aerobic conditions also appeared to be at least partially a function of salinity. Ectoine and glycine betaine accumulated between 0 and 30 g/L NaCl, and increased almost linearly with salinity. Total combined concentrations changed from 90 mM at 0 g/L NaCl, 190 mM at 20 g/L NaCl, and 260 mM at 30 g/L NaCl (Figure 10). In all cases, ectoine is the predominant solute comprising over 70% molar of total intracellular compatible solutes. At a moderate salinity (90 g/L NaCl), ectoine is the exclusive solute accumulated at levels of approximately 500 mM (Figure 8). As with the denitrifying samples, hydroxyectoine and ectoine are the sole solutes at the very highest salinity tested (175 g/L NaCl). The ratios of these two are similar to those found in denitrifying conditions: 320 mM ectoine and 30 mM hydroxyectoine (Figure 8). This further illustrates the cellular need for hydroxyectoine at the higher salinities as it is a more efficient osmotic protectant. Growth rates are suppressed at

higher salinities (0.01 h^{-1} at 175 g/L as opposed to an optimal 0.53 h^{-1} found at 20 g/L NaCl) (Figure 2).

This decrease in compatible solute accumulation is interesting and was not noted in other studies of *Halomonas* species. It may be that as the cells become increasingly stressed, energy is devoted to the most essential mechanisms of maintaining life. This may be a result of evolution to seasonal changes. As moisture evaporated from a saline system, *H. campisalis* may have evolved to begin saving its energy for a long dormant period. *Halomonas elongata* was noted to continually increase ectoine accumulation with increasing salinity, to a maximum of 123 mg/g of dry cells at a salinity of 150 g/L NaCl (Ono et al. 1998). However, *H. elongata* is not an alkaliphile. Being non-alkaliphilic may allow for the accumulation of greater levels of compatible solutes at high environmental ion concentrations without the interference of proton translocation necessary for alkaliphiles to maintain a neutral pH. As environmental salinity increases in the case of *H. campisalis*, however, there may be a greater need for intracellular Na^+ to overcome the gradient and maintain the ability to pump in protons. This increase in Na^+ would also negate the need for increasing compatible solute accumulation at the highest salinities in alkalihalophilic microorganisms.

As was observed with *H. campisalis*, hydroxyectoine is also produced solely at high salinities by *H. elongata* (Prabhu et al. 2004). Due to an additional hydroxyl group, hydroxyectoine is a more efficient osmotic protectant. This may be why it is produced toward the upper end of salinity, as well as why ectoine production drops at 175 g/L

NaCl. An ectoine hydroxylase gene, responsible for the conversion of ectoine to hydroxyectoine, has been identified in other halophiles (Prabhu et al. 2004) and this, or a similar gene may be present in *H. campisalis.* It may be, that this or related genes are expressed in the presence of high salinities, or by certain physiological stresses.

Ectoine, hydroxyectoine, and other compatible solutes have many applications as biomolecules, protectants, and stabilizers. For this consideration, it is important to examine total solute production, as well as intracellular accumulation. Since a direct link between solute accumulation and growth kinetics was not observed, the highest total solute production rate may not necessarily occur at the highest growth rate, the highest salinity, or at the highest intracellular levels of accumulation. In calculating total solute production within the growth medium, it was found that the concentration was the lowest in the absence of salt at 0.016 mM +/- 0.006 mM. This is reasonable since the growth rates are low and the lowest intracellular concentrations occurred under these conditions. When grown in a medium of 20 g/L NaCl, the concentration of ectoine was just slightly higher (0.019 mM +/- 0.011), likely due to the greater cell growth kinetics. The greatest levels of production occurred at 30 g/L NaCl and 90 g/L NaCl where concentrations were approximately 0.05 mM. Glycine betaine was produced at 0.01 mM +/- 0.006 mM at 90 g/L NaCl. Production dropped off to 0.017 +/- 0.007 mM ectoine and 0.007 mM +/- 0.001 hydroxyectoine in growth medium containing 175 g/L NaCl (Figure 9).
From this data, it appears, that under denitrifying conditions the optimal medium salinity for ectoine production is likely between 30 and 90 g/L NaCl.

As was the case with cultures grown under denitrifying conditions, intracellular compatible solute accumulation did not necessarily correlate directly to overall compatible solute production under aerobic conditions. Based on the volume of the entire medium, ectoine concentration was slightly greater than 0.02 mM in the absence of salinity, with much lower amounts of glycine betaine present. As opposed to intracellular accumulation, the changes in total compatible solute production were not linear with salinity, since increases in growth rates compounded increases in intracellular compatible solute accumulation. When grown in a medium containing 20 g/L NaCl, the medium concentration of ectoine was 0.05 mM +/- 0.013 mM and the glycine betaine levels were over 0.03 mM +/- 0.003 mM. At 30 g/L NaCl, the ectoine concentration reached 0.11 mM +/- 0.016 mM and the glycine betaine concentration did not change noticeably. At 90 g/L NaCl, the ectoine concentration was greater yet at 0.15 mM +/- 0.022 mM in the medium, while at 175 g/L NaCl, overall solute production dropped sharply (Figure 8).

Ectoine is currently produced commercially with a batch fermentation using *H. elongata*. This is an extreme halophile which is capable of producing ectoine at high levels of 102 mg ectoine/ g dry cell weight in an industrial setting (Ono et al. 1998). As reported here, ectoine can be produced under aerobic conditions at levels of 500 mM intracellularly. This translates to a production of about 43 mg ectoine/ g dry cell weight. Given that the substrate provided here (1 g/L glucose) could be cheaply increased, and optimal production of ectoine using *H. campisalis* may approach what can be produced using *H. elongata*. One additional benefit of the use of *H. campisalis* for ectoine production is the reduced salinities at which it thrives. Further investigation would be necessary to

optimize ectoine and hydroxyectoine production using *H. campisalis*. Current methods take advantage of bacterial milking, in which *H. elongata* is subjected to osmotic down-shock, causing it to release ectoine and hydroxyectoine into the media making for cheap and effective separation. The ability of *H. campisalis* to grow and produce ectoine at zero salinity may lead to a high yield using bacterial milking; in which several cycles of osmotic up-shock and down-shock could be efficiently used.

It can be seen that supplementing the growth medium with ectoine provided no significant increase in growth kinetics at 20 or 60 g/L NaCl (Figure 12). This may indicate that *H. campisalis* does not possess the capabilities to uptake ectoine from the environment. This would be expected as ectoine is predominantly synthesized within halophilic and halotolerant microorganisms. However, this is not shown definitively as ectoine may passively diffuse to and from the environment with no appreciable affect on the growth kinetics. It may be that additional accumulation of ectoine brings no discernable benefit to *H. campisalis* at these moderate salinities. No significant growth was seen when ectoine was supplied as the sole carbon source. This indicates that *H. campisalis* does not utilize ectoine as a carbon source. This is significant in that one reported use of compatible solutes is as intracellular carbon reserves (Oren 1999); clearly this was not observed for *H. campisalis*. Additionally, a link has been suggested between substrate utilization and uptake ability. Typically, halophiles that scavenge compatible solutes from the environment for osmotic protectants also possess the capability of utilizing them as a carbon source (Pfluger and Muller 2004). This would suggest that *H. campisalis* lacks an ectoine transport system. A similar test was performed with glycine

betaine. Again, *H. campisalis* did not utilize glycine betaine as the sole substrate. At the lowest salinity tested, 20 g/L NaCl, the presence of glycine betaine did not increase the maximum specific growth rate for *H. campisalis*. However, at 60 g/L, a slight increase in the maximum specific growth rate was observed (Figure 13). This indicates that *H. campisalis* may possess the necessary transport mechanisms to uptake glycine betaine from the environment, while lacking those necessary to utilize it as a carbon source. This is not at all unlikely as glycine betaine is commonly scavenged from the environment by a variety of halophilic microorganisms (Pfluger and Muller 2004). Additionally, it has been shown (Diaz 2005) that many transport systems may serve a dual purpose and are capable of scavenging glycine betaine.

Growth rate studies implementing varying substrate concentrations indicated a slightly greater need for higher substrate concentrations at increased salinity (Figures 3 through 7). While the overall maximum achievable growth rate changed little, the rate of return on increasing substrate increased slightly with salinity (Figure 7). This is not unexpected as more energy is needed for compatible solute synthesis at high salt conditions. A large substrate excess likely allows for the production and accumulation of additional compatible solutes.

PLFA Analysis - Significant differences in the PLFA structural groups were observed with changing salinity under both denitrifying and aerobic conditions. Notably, in the absence of salt there was a relative abundance of TBS fatty acids. Also, there was a

relative abundance of monoenoic fatty acids when cultures were grown in the presence of salinity.

Under aerobic and denitrifying conditions, the percent composition of normal saturate fatty acids was not observed to change significantly with changes in media salinities. This suggests that the synthesis of these fatty acids is not affected by changing salinity. It may also suggest that this type of fatty acid does not have a particular effect on the adaptation of *H. campisalis* to high or low salt conditions. The average fatty acid chain length was observed to increase with higher salinities. This has been observed in other studies of halophiles, one specific case by Brown et al. (2002) with *Oceanimonas baumannii*.

Under denitrifying conditions, the predominant TBS fatty acid was a15:0. All samples contained significant amounts of 18:1w7t fatty acids with monoenoic structures, with the exception of those grown at 30 g/L NaCl. These contained primarily 18:1w7c monoenoic fatty acids. This also is the salinity at which denitrifying samples showed the highest maximum specific growth rate. Holtwick et al. (1999) described the isomerization of trans fatty acids as a necessary adaptation by halophiles in a response to changes in salinity. The increase in trans fatty acids allows the cell membrane to maintain the correct level of fluidity when under stress from toxic compounds such as phenol or in the presence of large osmotic gradients. The lack of trans formations indicates that the cell wall has a minimal level of permeability. This may indicate that there is an optimum balance between environmental salinity and intracellular osmotic solutes. There were also

16:1w7c fatty acids found in all of the samples, though they did not make up a significant percent composition. In the absence of salinity, several types of monoenoic fatty acids were present in small amounts. There was an increase in overall fatty acid chain length as salinity increased. A decrease in chain lengths shorter than 18 carbons was offset by a marked increase in chain lengths of 18 carbons. As was the case with the monoenoic fatty acids, the cultures grown with no NaCl showed a wider variety of specific fatty acids in its phospholipid composition.

Interesting trends occurred within the monoenoic fatty acids in aerobically grown samples. While there were some cyclic fatty acids present in samples grown in the absence of salinity, the majority of all monoenoic fatty acids consisted of 18 carbon chains in the cis formation. Under aerobic conditions chain lengths were observed to increase with salinity, although not as markedly as under denitrifying conditions. This increase in chain length was limited to straight chained fatty acids, this is in contrast to a significant decrease in the large cyclic fatty acids with increasing salinity. Under both aerobic and denitrifying conditions, it appears that *H. campisalis* can change the PLFA composition of the cell membrane to adapt to changes in environmental salinity.

Certain PLFA composition patterns are known indicators of the physiological status of a culture. Specifically, the ratio of trans/cis fatty acids and the ratio of cyclic/cis structures were examined in this regard. Trans/cis structure ratios greater than 0.25 are a biological marker for adaptation in response to decreased membrane permeability. Under denitrifying conditions, a high trans/cis ratio of 2.41 was found in the sample grown at 0

g/L NaCl. This indicates that the cell is stressed by increasing membrane fluidity. Considering the compatible solute accumulation that was observed under zero salinity, it is possible that the presence of these solutes unbalanced by medium salinity is the cause. Ratios of 0.02, 0.02, 0.09, and 0.03 were found at 20, 30, 90, and 175 g/L NaCl, respectively. Typically, microorganisms increase their production of cyclopropyl groups under low substrate or toxic conditions. In these conditions, a low cell turnover rate exists, which leads to the formation of cyclic monoenoic acids. Cyclic formations are also known to increase membrane rigidity, decreasing permeability (Banciu et al. 2004). Cyclic fatty acids have been observed in *Halomonas salina* (Valderrama et al. 1998). In the case of *H. campisalis* relatively high cyclopropyl/cis ratio of 4.19 was observed in the absence of salinity. Much smaller ratios of 0.11, 0.21, 0.03, 0.11 were observed at 20, 30, 90, and 175 g/L NaCl (Figure 15).

The ratio of cyclopropyl to cis structures was also examined under aerobic conditions. The cyclo/cis ratios were observed with respect to increasing salinity as follows: 3.32, 0.11, 0.06, 0.43, and 0.36 (Figure 14). It appears that *H. campisalis* attempts to compensate for the lack osmotic balance in zero salinity by altering the PLFA composition (increasing trans and cyclic formation). The ratio of these conformations at the remaining salinities does not appear to follow a discernable pattern and are not considered large enough to have physiological significance. Of additional interest is the lack of cyclic fatty acids at very high salinities (175 g/L).

This is partly to be expected as the maximum specific growth rate in the absence of salinity was observed to be 0.35 h^{-1}, significantly lower than at 20 g/L NaCl (0.53 h^{-1}). However, it is surprising that the ratio of cyclic fatty acids is so large in the absence of salinity when compared with that found in samples grown at 90 g/L NaCl (0.43). The maximum specific growth rate at 90 g/L NaCl was observed to be 0.33 h^{-1}, nearly identical to that observed in the absence of salt. This indicates that cyclic fatty acids may not be solely indicative of cell stress in *H. campisalis*. Rather, growth of *H. campisalis* at low salinity suggests that this fatty acid structure may be an attempt by the cell to reduce cell membrane permeability encountered in the absence of salt. The ratio of trans to cis fatty acids was also largest in the absence of salinity with the aerobic samples as well. While a ratio of 1.51 was observed in samples grown with no salt, in the presence of salinity the trans/cis ratio never exceeded 0.04 (Figure 14). Given that growth kinetics indicate fairly robust growth in salt free media, the high ratios of trans/cis and cyclo/cis found in the samples grown at 0 g/L NaCl seem to be successful adaptations which may help *H. campisalis* grow in low salinities under both aerobic and denitrifying conditions.

If the PLFA data are considered with compatible solute accumulation results, conclusions for why cell permeability is prevalent in the absence of salinity can be drawn. Figures 10 and 11 showed that *H. campisalis* accumulated ectoine to cytoplasmic concentrations of 50 mM or higher in the absence of salinity under aerobic and denitrifying conditions; it is possible that a reverse osmotic gradient caused an increase in water flux into the cell membrane, leading to a high level of cell wall permeability. This may be cause for the depressed growth rates observed in the absence of salinity. This raises the possibility that

ectoine synthesis is not necessarily solely in response to environmental salinity. If this were the case, it would be expected that ectoine would not be found in *H. campisalis* in the absence of salinity.

One might expect some increase in cell wall permeability as growth rates are depressed at the higher salinities tested (175 g/L). The absence of high trans/cis ratios suggests that the high salinity may be hindering the growth of *H. campisalis* in a manner not related to osmotic pressure. Since *H. campisalis* is an alkaliphile, as well as a halophile, it relies on hydrogen ion pumps to maintain a neutral cytoplasmic pH. Typically either Na^+ ions or K^+ ions are pumped out of cells to allow for an influx of H^+ ions. As the Na^+ concentration in the medium is increased, it becomes more difficult for *H. campisalis* to maintain the necessary neutral pH within the cytoplasm. Although typically haloalkaliphiles maintain membranes that are impermeable to Na^+ and H^+ ions (Sudge et al. 1998), it may be that *H. campisalis* is only capable of optimizing the flux of these ions up to a certain salinity. PLFA analysis indicated that cell wall permeability increases slightly at 90 and 175 g/L NaCl. Given the size of ions and the high outside salinity, this may be enough to interfere with maintaining a neutral cytoplasmic pH at 175 g/L NaCl. Another possibility is that different transporters are used to facilitate this proton translocation. The genetic expression of one or more of these transporters may be hindered at salinities at or above 175 g/L NaCl. This was observed in the case of the haloalkaliphile *Vibrio cholerae* under saline conditions (Herz et al. 2002). The Na^+ and H^+ antiporters, NhaA and NhaB were necessary to Na^+ out of the cell and H^+ into the cell. NhaA was effective at high salinity, while NhaB was effective at low salinities. *H.*

campisalis may not lack a suitable antiporter at very high salinities. If this were the case, a neutral intracellular pH could not be maintained and the cell would not function properly at high salinities, despite maintaining a relatively impermeable membrane as is suggested by the PLFA analysis.

Of the three types of fatty acids: TBS, monoenoic, and normal saturates; the normal saturated fatty acids appeared to play the least role in adapting to environmental conditions since the sub-types and overall composition were observed to remain relatively constant through varying salinities under both denitrifying and aerobic environments. TBS fatty acids appeared only in the absence of salinity and are not produced in the presence of even low salt concentrations (20 g/L NaCl). Previous examples of this change in PLFA were not noted in a literature review by this author. This suggests that TBS fatty acids may play a role not yet understood in regulating membrane fluidity in halophiles. The monoenoic fatty acids take either the cis or trans formations, which are noted physiological markers for their effects on cell wall permeability. In addition, it is within the monoenoic fatty acid type that cyclic formations appear, another marker of cell stress.

Conclusions - The response of *H. campisalis* to changing salinity shows many similarities under aerobic and denitrifying conditions. However, it does appear that in some instances, electron acceptor availability may play a role in the effectiveness of the adaptations, as is the case in compatible solute accumulation.

With both O_2 and NO^-_3, terminally branched saturates are present only in the absence of salinity. Under both aerobic and denitrifying conditions, *H. campisalis* tended to produce longer straight chain fatty acids at higher salinities. Similar patterns have been noted with halophiles before (Brown et al. 2000). This increase in the phospholipid chain length may be an attempt to maintain the low permeability of the cell membrane as increasing salinity creates a greater osmotic pressure to drive water into the cell. With both electron acceptors, high levels of cyclic and trans fatty acids were present in the absence of salinity. In neither case does *H. campisalis* produce high levels of these conformations at the highest salinities where growth kinetics were depressed, indicating that cell wall permeability may not be a limiting factor for growth in these conditions. At high salinities, given that *H. campisalis* is an alkaliphile, growth may be limited by suppressed cation transport, limiting the cell's ability to maintain a neutral pH.

Similar patterns of growth kinetics and compatible solute production were observed in aerobic and denitrifying cultures. One significant difference was the higher growth rates, coupled with the higher compatible solute accumulations that occur under aerobic conditions. As discussed in the introduction, cells require energy to produce compatible solutes. The greater availability of energy under aerobic conditions likely is responsible for the increase in intracellular compatible solute production. Notably, compatible solutes did not accumulate as heavily in the absence of salinity, lending further evidence to the idea that cell wall permeability is a cause for cell stress.

Under both aerobic and anoxic conditions, hydroxyectoine was accumulated at high salinity (175 g/L). Given the similar ratios of ectoine and hydroxyectoine for aerobic and denitrifying conditions, it is likely the same mechanism is responsible for this conversion at high salinities under both aerobic and denitrifying conditions.

All studies reported here regarding *H. campisalis* have been performed in static condition batch reactors. Studies of dynamic adaptations to changes in salinity would be useful in determining the possibilities of a "milking" process to be used with *H. campisalis*. It would also be required to determine turn around time in batch fermentation. Additional studies should include a thorough study of protein expression to determine the role played by certain proteins and enzymes in cell adaptation and solute production and retention. Additionally, observations from these studies did not conclusively show that *H. campisalis* possessed the ability to utilize glycine betaine from the environment as a compatible solute. Additional studies carried out with higher medium salinity could shed light on this. Up-take of compatible solutes is energetically cheaper than synthesizing them *de novo* (Oren 1999). If *H. campisalis* has this ability it would be most apparent at the upper limits of growth, where the cell should be taxed for energy. A significant increase in growth rate should be seen if glycine betaine is available in the medium.

Chapter Three

Growth Kinetics of *Halobacterium. Salinarum* NRC-1

Abstract

The effects of salinity on the growth kinetics of the extremely halophilic archaeon *Halobacterium salinarum* strain NRC-1 are observed here. Parallel observations were made between aerobic and denitrifying conditions. An optimal growth rate of 0.047 h^{-1} at 200 g/L NaCl was seen with aerobic samples. Under denitrifying conditions the growth optima occurred at 150 g/L NaCl with a maximum specific growth rate of 0.041. Interestingly, along with the down-shift in optima growth salinity under denitrifying conditions, slight growth was observed at much lower salinities, even in the absence of salt under denitrifying conditions. In aerobic conditions no growth was detected below 150 g/L NaCl

3.1 – Introduction

Halophiles are organisms that have adapted to saline conditions, in some cases to such an extent that they may require high levels of sodium chloride or other salts to grow. One such example is the archaeon *Halobacterium,* which has been isolated both at the Dead Sea, and the Great Salt Lake. *Halobacterium* and other extremely halophilic archaea have modified the structure of their proteins and membranes to survive in saline conditions. These organisms accumulate high concentrations of potassium in order to remain hypertonic in respect to their surroundings; the internal potassium concentration may reach 4 to 7 molar (Prescott et al. 2003). The sodium ion acts to stabilize the plasma membrane of *Halobacterium*. If the concentration of sodium ions is too low, the cell wall and plasma membrane lose their structural integrity.

Representatives of archaea have long been considered to be limited to environmental extremes such as high salt, temperature or strict anoxia. The recent discovery of 16S rDNA gene sequences from archaea sources in water and soil samples has shed new light onto the possible capabilities archaea as well as the notion that they are obligate extremophiles (Purdy et al. 2004). Extremely halophilic archaea are typically found in salt and soda lakes, salt deposits and salterns, where they are typically the dominant heterotrophic organisms within their environment. All isolates reported to date are obligate extreme halophiles requiring a minimum of 9% salinity for significant growth, most however grow optimally between 20% and 26%, with some even growing well in saturated salt environments (Purdy et al. 2004). As suggested previously, archaea rely on

the “salt-in method for balancing osmolarity across the cell envelope. This evolution likely contributes toward a predominant narrow range in salinity (high salinities) at which these microorganisms thrive at. One such archaea is the extremely halophilic *Halobacterium Salinarum*, first isolated and sequenced by Ng et al (2000). This microorganism was selected for this work as it was well known and represented a typical and prevalent archaea. It was desired to test the patterns and differences in the growth kinetics of *H. salinarum* strain NRC-1 under varying salinities and with both aerobic and denitrifying conditions. The purpose was to surmise any effect the salt-in method may have when compared with the compatible solute method implemented by the halophilic bacteria *H. campisalis*.

3.2 – Materials & Methods

H. salinarum was grown on a basal mineral medium containing (in g/L) magnesium sulfate, 20; tri-sodium citrate, 3.0; potassium chloride, 2.0; tryptone, 5.0; yeast extract, 3.0; zinc sulfate, 0.000132; manganese sulfate, 0.000034; iron sulfate, 0.000078; cupric sulfate, 0.000014; sodium borate; 2. In addition sodium chloride was used as a salinity source and was added in amounts of between 0 and 250 g/L. Sodium chloride, magnesium sulfate, tri-sodium citrate, potassium chloride, sodium borate, tryptone, and yeast extract were added to distilled water in a 2 liter flask. The final volume was then adjusted to 1 liter using distilled water. The media was then autoclaved for 15 minutes at 121 degrees Celsius, and then allowed to cool to room temperature. A trace mineral solution containing the zinc sulfate, manganese sulfate, iron sulfate and cupric sulfate

dissolved in hydrochloric acid was then added to the media. The pH was then adjusted to 7.2 using 1 N hydrochloric acid. Original inoculate was supplied by Dr. DaSsarma of the University of Maryland in a frozen sample. Future inocula were preserved as glycerol-preserved samples (-80°C). For denitrifying samples, 0.5 g/L Sodium Nitrate was added to each sample flask. Cultures were cultivated through four growth phases prior to sampling. Aliquots of 6% (v/v) were transferred into fresh medium after the exponential growth phase had nearly ceased. After the fourth transfer, samples were taken to measure the cell density and determine the growth kinetics. Three replicate flasks were used for each experiment. Cultures were grown in 250-mL Erlenmeyer flasks (100 ml media volume) fitted with foam stoppers for the aerobic samples. Anaerobic serum bottles with rubber seals were used for the denitrifying samples. The denitrifying samples were purged with N_2 for 30 minutes to ensure an anaerobic environment. Both the aerobic and denitrifying samples were placed on shakers at 120 RMP and stored in an environmental chamber maintained at 37 degrees Celsius. An Eppendorf pipette (Westbury, NY) with a sterile tip (aerobic) and a Monoject 3cc Luer Lock Syringe with Polypropylene Hub Hypodermic Needles (denitrifying) were used to remove samples for cell growth analysis. Experiments were stopped after several consecutive measurements indicated that growth had ceased. Optical density measurements were taken at 595nm (45224 spectrophotometer Milton Roy Spectronic Genesys8453, St. Petersburg, FL). Un-inoculated medium was used to calibrate the spectrophotometer.

3.3 – Results

Under aerobic conditions *H. salinarum* grew with sodium chloride concentrations from 150 to 250 g/L NaCl, with a maximum specific growth rate of 0.047 h^{-1} +/- 0.004 h^{-1} occurring at 200 g/L NaCl (Figure 1 Appendix S). The growth rate rose rapidly from 150 to 200 g/L NaCl and then tapered off at 250 g/L NaCl. Under denitrifying conditions, optimum growth occurred at a NaCl concentration of 150 g/L with a maximum specific growth rate of 0.041 h^{-1} +/- 0.004 h^{-1} (Figure 1 Appendix T). Again the growth curve was sharp, rising quickly to the peak at 150 g/L NaCl and tailing off quickly. One difference standing out is the ability of *H. salinarum* to grow at very slow salinities under denitrifying conditions and even in the absence of salt. At salinities below 50 g/L a specific growth rate of approximately 0.004 h^{-1}. Growth is noticeably greater above 100 g/L NaCl.

3.4 – Discussion

All archaea from the genus *Halobacterium* rely on maintaining a high level of intracellular potassium chloride to balance the osmotic gradient. The salt-in method is a relatively energetically cheap method of combating saline stress; however it does require the cell divert some energy towards the process. Under denitrifying conditions less energy is available to the cells as the nitrate/glucose redox potential is less than that of oxygen/glucose. This may serve to explain the truncated growth curve under denitrifying conditions. As the cell requires energy for metabolism and the production of cellular

bodies, energy is diverted away from maintaining high levels of intracellular KCl. This would explain the down shift in the optimum growth rate from 200 g/L NaCl (aerobic) to 150 g/L NaCl (denitrifying). It may also explain why *H. salinarum* was able to grow, albeit frugally, at very low and zero salinity under denitrifying conditions. It is known that the intracellular enzymes and mechanisms of archaea are adapted to salt. Because of this they would not survive if the cytoplasm was salinity free. However, in this case there may have been such a shortage of energy that *H. salinarum* was not able to maintain KCl at concentrations where reverse osmotic pressure could damage the cell under low media salinities.

As is seen in Figure 1, appendix S, there is no growth when the medium salinity drops below 100 g/L NaCl in an aerobic environment. Given that it will grow in these conditions under the energy depressed denitrifying conditions, it is possible that intracellular salts are overly retained under energy rich conditions, suggesting that potassium and chlorine transporters are not sensitive to environmental conditions. Another possibility is that, unlike many intracellular enzymes and bodies in archaea, enzymes required for nitrate reduction in *H. salinarum* NRC-1 do not require high intracellular salt to function, allowing them to function in the absence of high environmental salinity.

This could be investigated by measuring intracellular KCl with mass spectrometry and documenting changes with medium salinity. This would confirm or rule out the possibility that reverse osmotic pressure is responsible for the decline in growth at low

salinities under denitrifying conditions, and for the lack of growth at low salinities under aerobic conditions. Identifying and monitoring the expression of genes responsible for coding enzymes and proteins that are responsible for potassium and chlorine flux under changing salinity would identify the effect of environmental cues on intracellular salt retention. As archaea are not generally well understood, this research could lead to an understanding in their survival methods and capabilities of adaptation. It has always been assumed that *H. salinarum* and other archaea have relatively narrow ranges of extreme conditions in which they can survive. It may be that through genetic engineering they may be used for a much wider range of applications in bioremediation and industry than what is currently believed. This possibility is supported by the recent isolation of haloarchaea from pore water sediments, were the salinity was only 4% (Purdy 2004). In addition haloarchaea have been recently isolated which have shown capable growth in environments below 2% salinity (Purdy 2004).

Chapter Four

Suggestions for Future Work

1. Further investigation would be necessary to optimize ectoine and hydroxyectoine production using *H. campisalis*. Current methods take advantage of bacterial milking, in which *H. elongata* is subjected to osmotic down-shock, causing it to release ectoine and hydroxyectoine into the media making for cheap and effective separation. All studies reported here regarding *H. campisalis* have been performed in static batch conditions. Studies of dynamic adaptations to changes in salinity would be useful in determining the possibilities of a "milking" process to be used with *H. campisalis*.

2. The compatible solute yield should be determined for bacterial milking. The ability of *H. campisalis* to grow in the absence of salinity may increase the percent yield of compatible solutes while allowing for several high-yield cycles of milking to be carried out consecutively.

3. The identification of key proteins and the salinities at which they are expressed would mark genes responsible for adaptations to osmotic stress. This knowledge could lead to the optimization of ectoine or hydroxyectoine production.

4. Compatible solutes have been identified as thermoprotectants (Oren 1999) as well. An increase in temperature of the growth medium may significantly increase production and accumulation of compatible solutes. Compatible solute type and quantity should be analyzed as a function of temperature at which *H. campisalis* will grow.

References

1. Albers S, Van de Vossenberg J, Driessen A, Konings W (2001) Bioenergetics and solute uptake under extreme conditions. Extremophiles 5:285-294

2. Alva V, Peyton B (2003) Phenol and catechol biodegradation by the haloalkaliphile *Halomonas campisalis*: influence of pH and salinity. Environmental Science and Technology 19:4397-4402

3. Arzumanyan VG, Voronina NA, Geidebrekht OV, Shelemekh OV, Plakunov VK, Belyaev SS (2001) Antagonistic Interactions between Stress Factors during the Growth of Microorganisms under Conditions Simulating the Parameters of Their Natural Ecotopes. Microbiology 71:133-138

4. Aspe E, Marti MC, Roeckel M (1997) Anaerobic treatment of fishery wastewater using a marine sediment inoculum. Water Research 31:2147-2160

5. Bayley RM, Morton RA (1978) Recent developments in the molecular biology of extremely halophilic bacteria. Critical Revolutionary Microbiology 6:151-205

6. Banciu H, Sorokin D, Rijpstra I, Damste J, Galinski E, Takaichi S, Muyzer G, Kuenen G (2005) Fatty acid, compatible solute andpigment composition of obligately chemolithoautotrophic alkaliphilic sulfur-oxidizing bacteria from soda lakes. FEMS Microbiology Letters 243:181-187

7. Bastos AER, Moon DH, Rossi A, Trevors JT, Tsai SM (2000) Salt-tolerant phenol-degrading microorganisms isolated from Amazonian soil samples. Arch Microbiology 174:346-352

8. Boltyanskaya Y, Detkova EN, Shumskii AN, Duluv LE, Pusheva MA Osmoadaptation in Representatives of Haloalkaliphilic Bacteria from Soda Lakes. Microbiology 74:640-645

9. Boltyanskaya Y, Antipov AN, Kolganova TV, Lysenko AM, Kostrikina NA, Zhilina TN (2004) *Halomonas campisalis*, an Obligatorily Alkaliphilic, Nitrous Oxide-Reducing Denitrifier with a Molybdenum Dofactor-Lacking Nitrate Reductase. Microbiology 73:271-278

10. Brown G, Sutcliffe Iain, Bendell D, Cumings S (2000) The modification of the membrane of *Oceanomonas baumannii* when subjected to both osmotic and organic solvent stress. FEMS Microbiology Letters 189:149-154

11. Brown G, Sutcliffe I, Cummings S (2003) Combined solvent and water activity stresses on turgor regulation and membrane adaptation in *Oceanimonas baumannii* ATCC 700832. *Antonie van Leeuwenhoek* 83: 275-283

12. Brzostowicz PC, Gibson KL, Thomas SM, Blasko MS, Rouviere PE (2000) Simultaneous identification of two cyclohexanone oxidation genes from an environmental *Brevibacterium* isolate using mRNA differential display. Journal of Bacteriology 182:4241-4248

13. Cayol JL, Ollivier B, Patel BKC, Prensier G, Guezennec J, Garcia JL (1994) Isolation and characterization of *Halothrmothrix orennii* gen. nov., sp. Nov., a halophilic, thermophilic, fermentative, strictly anaerobic bacterium. Int J Syst Bacteriol 44:534-540

14. Davis, Greg, Personal Communication, Microbial Insights (2006).

15. Delille D, Basseres A, Dessommes AA (1998) Effectiveness of bioremediation for oil-polluted Antarctic seawater. Polar Biology 19:237-241

16. Diaz MR, Taylor BF (1996) Metabolism of methylated osmolytes y aerobic bacteria from Mono Lake, a moderately hypersaline, alkaline environment. FEMS Microbiology Ecology 19:239-247

17. DoAaz Mp, Boyd KG, Grigson SJW, Burgess JG (2002) Biodegradation of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M, immobilized onto polypropylene fibers. Biotechnology and bioengineering 79:145-153

18. Duckworth A, Grant W, Jones B, Meijer D, Marquez MC, Ventosa A (2000) *Halomonas magadii* sp. Nov., a new member of the genus *Halomonas*, isolated from a soda lake of the East African Rift Valley. Extremophiles 4:53-60

19. Fischel U, Oren A (1993) Fate of compatible solutes during dilution stress in *Ectothiorodospira marismortui*. FEMS Microbiology Letters 113:113-118

20. Galinski E (1993) Compatible solutes of halophilic eubacteria: molecular principles, water-solute interaction, stress protection. Experientia 49:487-496

21. Galinski E (1995) Osmoadaptation in bacteria. Advanced Microbial Physiology 37:273-328

22. Galinski E, Beckmann M, Kunte J, Severin J (1997) Recovery of individual isomers from mixtures using cells or microorganisms. Patent DE19622168

23. Galinski E, Oren A (1991) Isolation and structure determination of a novel compatible solute from the moderately halophilic purple sulfur bacterium

Ectothiorhodospira marismortui. European Journal of Biochemistry 198:593-598

24. Galinski E, Pfeiffer HP, Truper HG (1985) 1,4,5,6-Tetrahydro-2-methyl-4-pyrimidinecarboxylic acid: A novel cyclic amino acid from halophilic phototrophic bacteria of the genus *Ectothiorhodospira*. European Journal of Biochemistry 149:135-139

25. Galinski E, Truper HG (1982) Betaine, a compatible solute in the extremely halophilic phototrophic bacterium *Ectothiorhodospira halochloris*. FEMS Microbiology Letters 13:356-360.

26. Galinski E, Truper HG (1994) Microbial behavior in salt-stressed ecosystems. FEMS Microbiology Review 15:95-108.

27. Garty H, Caplan SR (1977) Light-dependent rubidium transport in intact *Halobacterium halobium* cells. Biochem., Biophys. Acta 459: 532-545

28. Grammann K, Volke A, Kunte HJ (2002) New Type of Osmoregulated Solute Transporter Identified in Halophilic Members of the Bacteria Domain: TRAP Transporter TeaABC Mediates Uptake of Ectoine and Hydroxyectoine. Journal of Bacteriology 3078-3085.

29. Gruber C, Legat A, Pfaffenhuemer M, Radax C, Weidler G, Busse HJ, Stan-Lotter H (2004) *Halobacterium noricense* sp. Nov., an archaeal isolate from a bore core of an alpine Permian salt deposit, classification of *Halobacterium* sp. NRC-1 as a strain of *H. salinarum* and emended description of *H. salinarum*. Extremophiles 8:431-439

30. Herbst, D (1998) Potential salinity limitations on nitrogen fixation in sediments from Mono Lake, California. International Journal of Salt Lake Research 7:261-274

31. Herz K, Vimont S, Padan E, Berche P (2003) Roles of NhaA, NhaB, and NhaD Na+/H+ Antiporters in Survival of *Vibrio cholerae* in a Saline Environment. Journal of Bacteriology February 1236-1244

32. Hezayen FF, Rehm BHA, Eberhardt R, Steinbuchel A (2000) Polymer production by two newly isolated extremely halophilic archaea: application of a novel corrosion-resistant bioreactor. Applied Microbiology Biotechnology 54:319-325

33. Hinteregger C, Streichsbier F (1997) *Halomonas* sp., a moderately halophilic strain, for biotreatment of saline phenolic waste-water. Biotechnology Letters November 1099-1102

34. Holtwick R, Keweloh H, Meinhardt F (1999) *cis/trans* Isomearse of Unsaturated Fatty Acids of *Pseudomonas putida* P8: Evidence for a Heme Protein of the Cytochrome *c* Type. Applied and Environmental Microbiology 65:2644-2649

35. Jackson WA, Pardue JH (1999) Potential for enhancement of biodegradation of crude oil in Louisiana salt marshes using nutrient amendments. Water Air Soil Pollution 109:343-355

36. Jackson WA, Pardue JH (1997) Seasonal variability of crude oil respiration potential in salt and fresh marshes. Journal of Environmental Quality 26:1140-1146

37. Jebbar M, Talibart R, Gloux K, Bernard T, Blanco C (1992) Osmoprotection of *Escherichia coli* by ectoine: Uptake and accumulation characteristics. Journal Bacteriology 174:5027-5035

38. Kargi F, Uygur A (1996) Biological treatment of saline wastewater in an aerated percolator unit utilizing halophilic bacteria. Environmental Technology 17:325-330

39. Kraegeloh A, Kunte HJ (2002) Novel insights into the role of potassium for osmoregulation in *Halomonas elongata*. Extremophiles 6:453-462

40. Krulwich TA, Ito M, Hicks DB, Gilmour R, Guffanti AA (1998) pH homeostatis and ATP synthesis: studies of two processes that necessitate inward proton
translocation in extremely alkaliphilic *Bacillus* species. Extremophiles 2:217-222

41. Kushner DJ (1978) Life in high salt and solute concentrations: halophilic bacteria. Academic Press London 317-368

42. Kuznetsov VD, Zaitseva TA, Bakulenko LV, Filippova SN (1992) *Streptomyces albiaxalis* sp. Nov.: a new petroleum hydrocarbon-degrading species of thermo- and halotolerant *Streptomyces*. Microbiology 61:62-67

43. Lippi D, Paolis MR, Mattia ED, Grego S, Pietrosanti T, Cacciari I (2000) Effect of salinity on growth and starvation-survival of a tropical *Rhizobium* strain. Biology and Fertil Soils 30:276-283

44. Mackay MA, Norton RS, Borowitzka LJ (1984) Organic osmoregulatory solutes in cyanobacteria. Journal of General Microbiology 130:2177-2191

45. Madern D, Ebel C, Zaccai G (2000) Halophilic adaptation of enzymes. Extremophiles 4:91-98

46. Maltseva O, Oriel P (1997) Monitoring of an alkaline 2,4,6-trichlorophenol-degrading enrichment culture by DNA fingerprinting methods and isolation of the responsible organism, haloalkaliphilic *Nocardioides* sp. Strains M6. Applied Environmental Microbiology 63:4145-4149

47. Margesin R, Schinner F (2001) Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles 5:73-83.

48. Maskow T, Klensteuber S (2004) Carbon and energy fluxes during haloadaptation of *Halomonas* sp. EF11 growing on phenol. Extremophiles 8:133-141

49. Monteoliva-Sanchez M, Ferrer MR, Ramos-Cormenzana A, Quesada E, Monteoliva M (1988) Cellular fatty acid composition of *Deleya halophile* – effect of growth temperature and salt concentration. Journal of General Microbiology 134:199-203

50. Motitschke L, Driller H, Galinski E (2000) Ectoine and ectoine derivatives as moisturizers in cosmetics. Patent US060071

51. Mojica FJM, Cisneros E, Ferrer C, Rodriguez-Valera F, Juez, G (1997) Osmotically Induced Response in Representatives of Halophilic Prokaryotes: the Bacterium *Halomonas elongate* and the Archaeon *Hloferax volcanii*. Journal of Bacteriology September 5471-5481

52. Mormile M, Romine M, Garcia T, Ventosa A, Bailey T, Peyton B (1999) *Halomonas campisalis* sp. Nov., a Denitrifying, Moderately Haloalkaliphilic Bacterium. System of Applied Microbiology 22: 551-558

53. Muller V, Oren A (2003) Metabolism of chloride in halophilic prokaryotes. Extremophiles 7:261-266

54. Ng WV et al. (2000) Genome sequence of *Halobacterium* species NRC-1. National Academy of Sciences USA 97:12176-12181

55. Nicolaus B, Manca MC, Lama L, Esposito E, Gambacorta A (2001) Lipid modulation by environmental stresses in two models of extremophiles isolated from Antarctica. Polar Biology 24:1-8

56. Nicolaus B, Moriello, VS, Lama, L, Poli, A, Gambacorta A (2003) Polysaccharides From Extremophilic Microorganisms. Origins of Life and Evolution of the Biosphere 34: 159-169

57. Oesterhelt D, Patzelt H, Kesler B (1998) Decomosition of halogenerated hydrocarbons by halophilic bacteria. Patent DE19639894.

58. Ono H, Okudo M, Tongpim S, Imai K, Shinmyo A, Sakuda S, Kaneko Y, Murooka Y, Takano M (1998) Accumulation of Compatible Solutes, Ectoine and Hydroxyectoine, in a Moderate Halophile, *Halomonas elongata* KS3 Isolated from Dry Salty Land in Thailand. Journal of Fermentation and Bioengineering 85:362-368

59. Oren A (1999) Bioenergetic Aspects of Halophilism. Microbiology and Molecular Biology Reviews 63:334-338

60. Oren A (2002) Diversity of halophilic microorganisms: Environments, phylogeny, physiology, and applications. Journal of Industrial Microbiology & Biotechnology 28:56-63

61. Oren A (1986) Intracellular salt concentrations of the anaerobic halophilic eubacteria *Haloanaerobium praevalens* and *Halobacteroides halobius*. Canadian Journal of Microbiology 32:4-9

62. Oren, A (2002) Molecular ecology of extremely halophilic Archaea and Bacteria. FEMS Microbiology Ecology 39:1-7

63. Oren A (2000) Salts and Brines: Ecology of Cyanobacteria: Their diversity in Time and Space. Kluwer Academic Publishers 281-306

64. Oren, A (2001) The bioenergetic basis for the decrease in metabolic diversity at increasing salt concentrations: implications for the functioning of salt lake ecosystems. Hydrobiology 466: 61-72

65. Oren A (1994) The ecology of extremely halophilic Archaea. FEMS Microbiology Review 13:415-440

66. Oren A (1995) The role of glycerol in the nutrition of halophilic archaeal communities: a study of respiratory electron transport. FEMS Microbiology Ecology 16:281-290

67. Oren A, Gurevich P, Azachi M, Hents Y (1992) Microbial degradation of pollutants at high salt concentrations. Biodegradation 3:387-398

68. Oren A, Heldal M, Norland S, Galinski E (2002) Intracellular ion and organic solute concentrations of the extremely halophilic bacterium *Salinibacter rubber*. Extremophiles (2002) 6:491-498

69. Oren A, Litchfield C (1999) A procedure for the enrichment and isolation of *Halobacterium*. FEMS Microbiology Letters 173:353-358

70. Pankhurst CE, Yu S, Hawke BG, Harch BD (2001) Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil

microbial communities associated with increased salinity or alkalinity at three locations in South Australia. Biology and Fertile Soils 33:204-217

71. Pfluger K, Muller V (2004) Transport of Compatible Solutes in Extremophiles. Journal of Bioenergetics and Biomembranes 36:17-24

72. Prabhu J, Schauwecker F, Grammel N, Keller U, Bernhard M (2004) Functional Expression of the Ectoine Hydroxylase Gene (thpD) from *Streptomyces chryomallus* in *Halomans elongate*. Applied and Environmental Microbiology May 3130-3132

73. Prescott, L; Harley, J; Klein, Donald. Microbiology. McGraw Hill, 5th Ed. 2003. 122-125

74. Purdy KJ, Cresswell-Maynard TD, Nedwell DB, McGenity TJ, Grant WD, Timmis KN Embley TM (2004) Isolation of haloarchaea that grow at low salinities. Environmental Microbiology 6:591-595

75. Roberts M (2000) Osmoadaptation and osmoregulation in archaea. Frontiers in Bioscience 5:796-812

76. Roebler M, Muller V (2002) Chloride, a New Environmental Signal Molecule Involved in Gene Regulation in a Moderately Halophilic Bacterium, *Halobacillus halophilus*. Journal of Bacteriology November 6207-6215

77. Russell NJ (1989) Adaptive modifications in membranes of halotolerant and halophilic microorganisms. Journal of Bioenergy and Biomembranes 21:93-113

78. Russel NJ, Kogut M, Kates M (1985) Phospholipid biosynthesis in the moderately halophilic bacterium *Vibrio costicola* during adaptation to changing salt concentrations. Journal of General Microbiology 131:781-789.

79. Sanchez-Porro C, Mellado E, Bertoldo C, Antranikian G, Ventosa A (2003) Screening and characterization of the protease CP1 produced by the moderately halophilic bacterium *Pseudoalteromonas* sp. Strain CP76

80. Sauer T, Galinski E (1998) Bacterial milking: a novel bioprocess for production of compatible solutes. Biotechnology Bioengineering 57:306-313

81. Silva Z, Borges N, Martins LO, Wait R, Costa MSD, Santos H (1999) Combined effect of the growth temperature and salinity of the medium on the accumlationof compatible solutes by *Rhodothermus marinus* and *Rhodothermus obamensis*. Extemophiles 3:163-172

82. Sorokin D, Banciu H, Loosdrecht M, Kuenen JG (2003) Growth physiology and competitive interaction of obligately chemolithoautotrophic, haloalkaliphilic, sulfur-oxidizing bacteria from soda lakes. Extremophiles 7:195-203

83. Steward GF, Zehr JP, Jellison R, Montoya JP, Hollibaugh JT (2004) Vertical Distribution of Nitrogen-Fixing Phylotypes in a Meromictic, Hypersaline Lake. Microbial Ecology 47:30-40

84. Stouthamer AH (1973) A theoretical study on the amount of ATP required for synthesis of microbial cell material. Antonie Leeuwenhoek 39:545-565

85. Sudge SS, Bastawde KB, Gokhale DV, Kalkote UR, Ravindranathan T (1998) Production of D-hydantoinase by halophilic *Pseudomonas* sp. NCIM 5109. Applied Microbiology & Biotechnology 49:594-599.

86. Sutton GC, Russel NJ, Quinn PJ (1991) The effect of salinity on the phase behavior of total lipid extracts and binary mixtures of the major phospholipids isolated from a moderately halophilic eubacterium. Biochemistry and Biophysics 1061: 235-246

87. Toyoda Y, Oowaya K, Takano M, Shibata S (1997) Stabilization of enzyme. Patent JP9143167

88. Valderrama MJ, Monteooliva-Sanchez M, Quesada E, Ramos-Cormenzana A (1998) Influence of salt concentration on the cellular fatty acid composition of the moderately halophilic bacterium *Halomonas salina*. 149:675-679

89. Van de Vossenberg J, Driessen AJM, Grant W, Konings W (1999) Lipid membranes from halophilic and alkali-halophilic Archaea have a low H+ and Na+ permeability at high salt concentration. Extremophiles 3:253-257

90. Van de Vossenberg J, Ubbink-Kok T, Elferink MGL, Driessen AJM, Konings W (1995) Ion permeability of the cytoplasmic membrane limits the maximum growth temperature of bacteria and archaea. Molecular Microbiology 18(5):925-932

91. Ventosa A, Nieto JJ, Oren A (1998) Biology of moderately halophilic aerobic bacteria. Microbiology and Molecular Biology Review 62:504-544

92. Victor W et al (2000) GenomeSequence of *Halobacterium* species NRC-1. PNAS 97:12176-12181

93. Walsby AE (1971) The pressure relationships of gas vacuoles. Proc. R. Soc. London 178:301-326

94. Wegmann K, Ben-Amotz A, Avron M (1980) Effect of temperature on glycerol retention in the halotolerant algae *Dunaliella* and *Asteromonas*. Plant Physiology 66:1196-1197

95. Welsh D, (2000) Ecological significance of compatible solute accumulation by microorganisms: from single cells to global climate. FEMS Microbiology Reviews 24:263-290

96. Wright P, Tanaka T (2002) Physiological modeling of the response of *Kocuria rosea* exposed to changing water activity. Biotechnology Letters 24:603-609

97. Woolard CR, Irvine RL (1994) Biological treatment of hypersaline wastewater by abiofilm of halophilic bacteria. Water Environmental Resources 66:230-235

98. Zhilina TN, Garnova ES, Tourova TP, Kostrikina NA, Zavarzin GA (2001) *Halonatronum saccharophilum* gen. nov. sp. No.: A New Haloalkaliphilic Bacterium of the Order *Haloanaerobiales* from Lake Magadi. Microbiology 70(1): 77-85

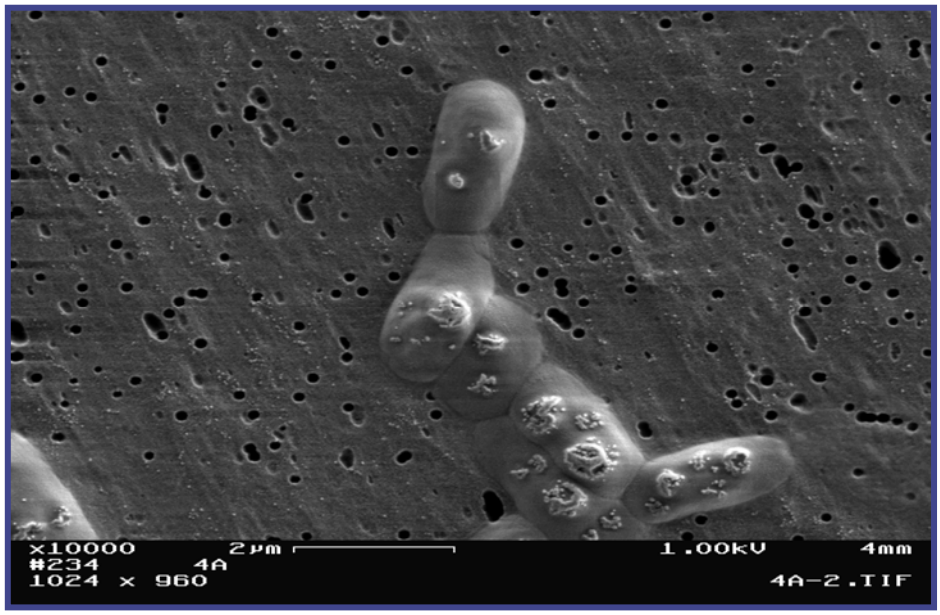
99. Zyyagintseva IS, Belyaev SS, Borzenkov IA, Kostrikina NA, Milekhina EI, Ivanov MV (1995) Halophilic archaebacteria from the Kalamkass oil field. Microbiology 64:67-71

Appendices

Appendix A

This appendix presents physical characteristics of *H. campisalis*. Because growth kinetics were determined using optical density, a correlation relating optical density at 595 nm with dry cell weight is also included.

Characteristics of *H. campisalis*



Gram Negative Bacteria

Moderate Alkaliphile

Moderate Halophile

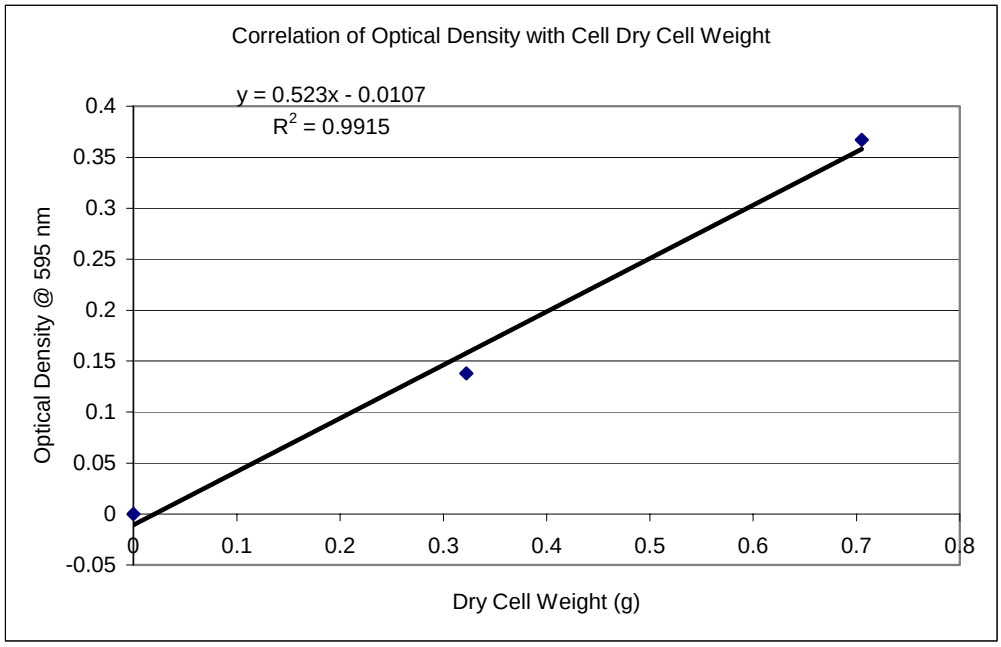
Forms white circular colonies with smooth edges

Motile rod

3-5 µm x 1 µm in dimension

G+C content ~ 66 mol%

Cell Wt. (g/L)	Absorbance
0	0
0.322	0.138
0.705	0.367



Appendix B

Appendix B presents the growth medium used to cultivate *H. campisalis*. Substrate (Glucose and sodium nitrate) were not included in this table as they varied from aerobic to denitrifying conditions.

Growth medium for *H. campisalis*

For 1 Liter Media

Sodium Chloride	0-260 g/L
Sodium Borate	4 g/L
Ammonium Chloride	1 g/L
Potassium Phosphate	0.5 g/L

Add Distilled Water to 1 Liter

Autoclave @ 121 degrees Celsius for 15 min
Allow to cool to room temperature

Adjust pH using HCl or NaOH
Add mineral media

Mineral media for campisalis in mg/L

NaNO3	10
Na2SO4	10
CoCl2*H2O	0.12
MnCl2*4H2O	0.10
ZnCl2	0.07
NiCl2*6H2O	0.025
Na2MoO4*2H2O	0.025
CuCl2*2H2O	0.15
MgCl2	60
CaCl2	50

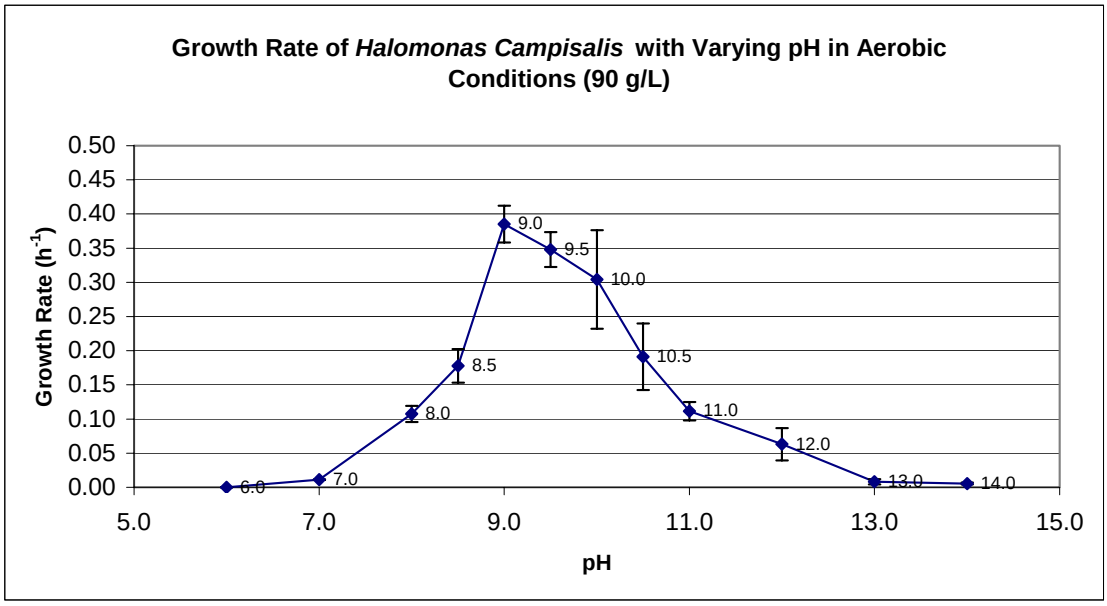
Appendix C

Appendix C presents optical density data gathered for the growth of *H. campisalis* over a wide range of pH in the growth medium. All experiments were performed in the presence of 90 g/L NaCl, as this was the salinity at which growth optima was earlier reported to occur at. The maximum specific growth rate was determined by plotting the natural logarithms of the optical density data versus elapsed growth time. A linear line was then fit to the steepest part of this data set. The slope of this line represented the maximum specific growth rate.

Growth kinetics for *H. campisalis* with varying pH (aerobic conditions)

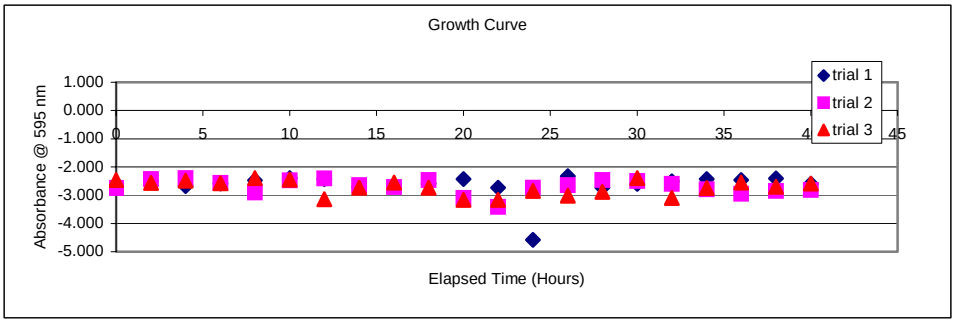
Total pH data

pH	Growth Rate h-1	Standard Derivations
6.0	0.0000	0.0000
7.0	0.0112	0.0004
8.0	0.1075	0.0118
8.5	0.1779	0.0246
9.0	0.3852	0.0270
9.5	0.3480	0.0256
10.0	0.3042	0.0721
10.5	0.1911	0.0489
11.0	0.1114	0.0135
12.0	0.0630	0.0237
13.0	0.0082	0.0038
14.0	0.0056	0.0015



pH 6

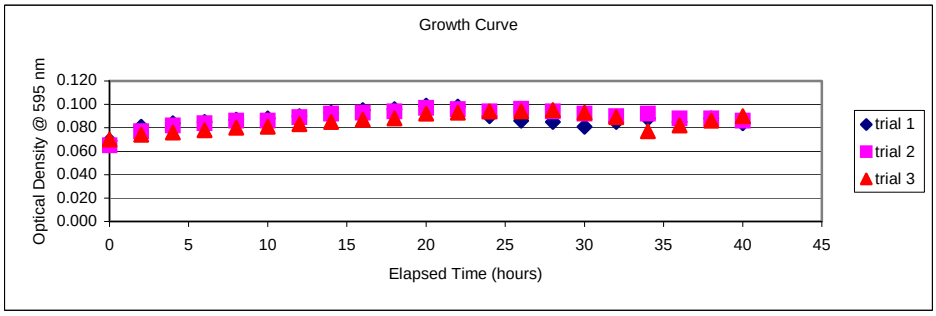
				Logarithms		
Elapsed Time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.074	0.064	0.085	-2.604	-2.749	-2.465
2	0.081	0.088	0.077	-2.513	-2.430	-2.564
4	0.069	0.091	0.083	-2.674	-2.397	-2.489
6	0.075	0.077	0.076	-2.590	-2.564	-2.577
8	0.084	0.055	0.091	-2.477	-2.900	-2.397
10	0.091	0.084	0.086	-2.397	-2.477	-2.453
12	0.088	0.090	0.043	-2.430	-2.408	-3.147
14	0.066	0.071	0.065	-2.718	-2.645	-2.733
16	0.071	0.066	0.078	-2.645	-2.718	-2.551
18	0.075	0.085	0.065	-2.590	-2.465	-2.733
20	0.088	0.045	0.042	-2.430	-3.101	-3.170
22	0.065	0.033	0.042	-2.733	-3.411	-3.170
24	0.010	0.065	0.058	-4.585	-2.733	-2.847
26	0.098	0.071	0.049	-2.323	-2.645	-3.016
28	0.065	0.085	0.056	-2.733	-2.465	-2.882
30	0.074	0.082	0.091	-2.604	-2.501	-2.397
32	0.081	0.074	0.045	-2.513	-2.604	-3.101
34	0.088	0.061	0.064	-2.430	-2.797	-2.749
36	0.085	0.052	0.078	-2.465	-2.957	-2.551
38	0.090	0.058	0.067	-2.408	-2.847	-2.703
40	0.074	0.060	0.075	-2.604	-2.813	-2.590

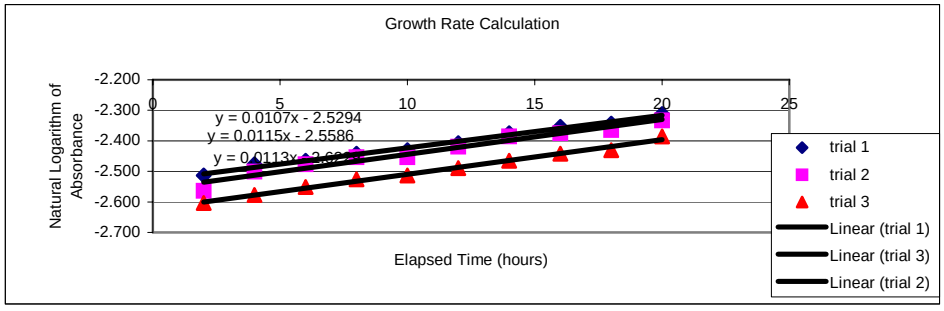


	Growth Rate			
trial 1	trial 2	trial 3	Average	STD
0.000	0.000	0.000	0.0000	0.0000

pH 7

				Logarithms		
Elapsed Time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.069	0.065	0.070	-2.674	-2.733	-2.659
2	0.081	0.077	0.074	-2.513	-2.564	-2.604
4	0.084	0.082	0.076	-2.477	-2.501	-2.577
6	0.085	0.084	0.078	-2.465	-2.477	-2.551
8	0.087	0.086	0.080	-2.442	-2.453	-2.526
10	0.088	0.086	0.081	-2.430	-2.453	-2.513
12	0.090	0.089	0.083	-2.408	-2.419	-2.489
14	0.093	0.092	0.085	-2.375	-2.386	-2.465
16	0.095	0.093	0.087	-2.354	-2.375	-2.442
18	0.096	0.094	0.088	-2.343	-2.364	-2.430
20	0.099	0.097	0.092	-2.313	-2.333	-2.386
22	0.098	0.096	0.093	-2.323	-2.343	-2.375
24	0.090	0.094	0.094	-2.408	-2.364	-2.364
26	0.086	0.096	0.094	-2.453	-2.340	-2.364
28	0.085	0.094	0.095	-2.465	-2.364	-2.354
30	0.081	0.092	0.093	-2.513	-2.386	-2.375
32	0.085	0.090	0.089	-2.465	-2.408	-2.419
34	0.088	0.092	0.077	-2.430	-2.386	-2.564
36	0.085	0.088	0.082	-2.465	-2.430	-2.501
38	0.088	0.088	0.086	-2.430	-2.430	-2.453
40	0.084	0.086	0.090	-2.477	-2.453	-2.408

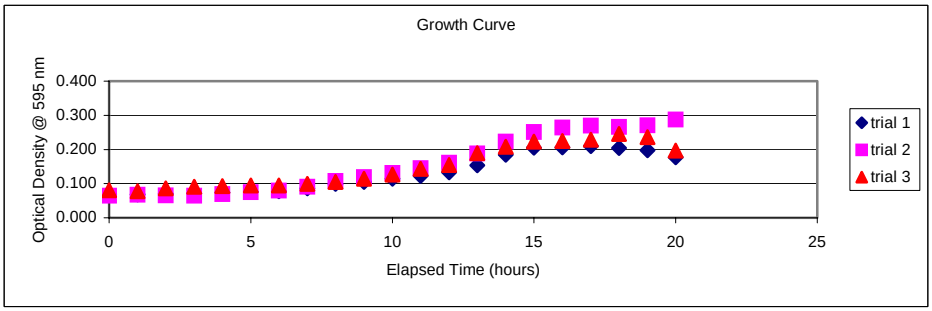


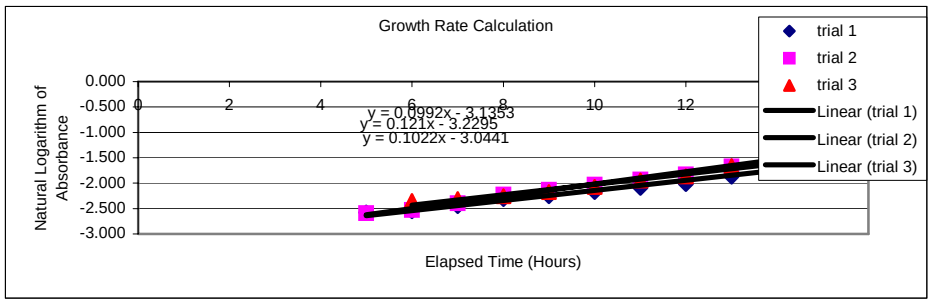


	Growth Rate			
trial 1	trial 2	trial 3	Average	STD
0.011	0.012	0.011	0.0112	0.0004

pH 8

					Logarithms	
Elapsed Time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.069	0.065	0.081	-2.674	-2.733	-2.513
1	0.068	0.067	0.078	-2.688	-2.703	-2.551
2	0.070	0.066	0.086	-2.659	-2.718	-2.453
3	0.071	0.065	0.091	-2.645	-2.733	-2.397
4	0.074	0.069	0.093	-2.604	-2.674	-2.375
5	0.076	0.075	0.095	-2.577	-2.590	-2.354
6	0.078	0.080	0.095	-2.551	-2.526	-2.354
7	0.086	0.091	0.099	-2.453	-2.397	-2.313
8	0.099	0.108	0.105	-2.313	-2.226	-2.254
9	0.105	0.119	0.114	-2.254	-2.129	-2.172
10	0.114	0.131	0.126	-2.172	-2.033	-2.071
11	0.123	0.145	0.143	-2.096	-1.931	-1.945
12	0.133	0.161	0.155	-2.017	-1.826	-1.864
13	0.154	0.188	0.189	-1.871	-1.671	-1.666
14	0.185	0.223	0.208	-1.687	-1.501	-1.570
15	0.205	0.251	0.223	-1.585	-1.382	-1.501
16	0.206	0.264	0.225	-1.580	-1.332	-1.492
17	0.210	0.270	0.229	-1.561	-1.309	-1.474
18	0.204	0.266	0.245	-1.590	-1.324	-1.406
19	0.198	0.271	0.236	-1.619	-1.306	-1.444
20	0.177	0.288	0.197	-1.732	-1.245	-1.625

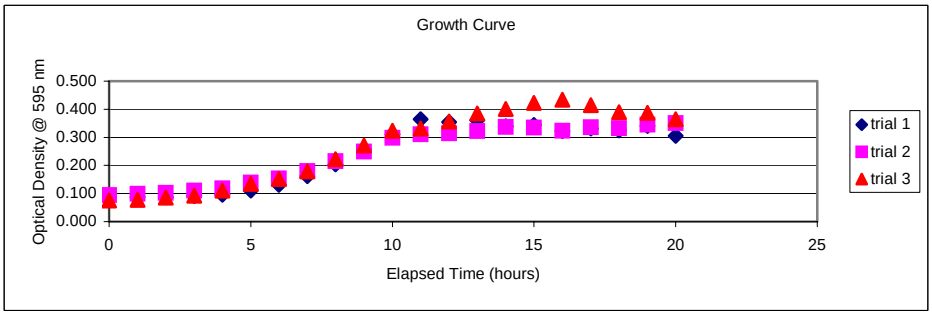


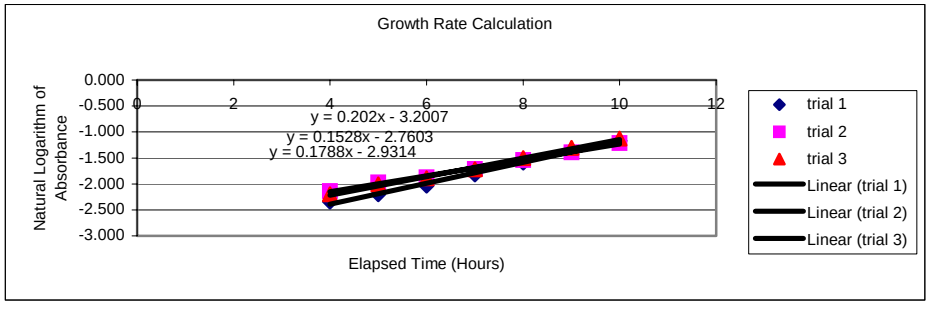


	Growth Rate			
trial 1	trial 2	trial 3	Average	STD
0.0992	0.1210	0.1022	0.1075	0.0118

pH 8.5

				Logarithms		
Elapsed Time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.086	0.095	0.075	-2.453	-2.354	-2.590
1	0.088	0.099	0.078	-2.430	-2.313	-2.551
2	0.087	0.103	0.085	-2.442	-2.273	-2.465
3	0.091	0.110	0.092	-2.397	-2.207	-2.386
4	0.096	0.118	0.111	-2.343	-2.137	-2.198
5	0.111	0.139	0.134	-2.198	-1.973	-2.010
6	0.131	0.154	0.151	-2.033	-1.871	-1.890
7	0.162	0.180	0.179	-1.820	-1.715	-1.720
8	0.204	0.215	0.222	-1.590	-1.537	-1.505
9	0.258	0.249	0.271	-1.355	-1.390	-1.306
10	0.311	0.298	0.324	-1.168	-1.211	-1.127
11	0.364	0.311	0.331	-1.011	-1.168	-1.106
12	0.354	0.314	0.356	-1.038	-1.158	-1.033
13	0.361	0.322	0.385	-1.019	-1.133	-0.955
14	0.339	0.337	0.401	-1.082	-1.088	-0.914
15	0.344	0.335	0.422	-1.067	-1.094	-0.863
16	0.322	0.324	0.434	-1.133	-1.127	-0.835
17	0.331	0.336	0.415	-1.106	-1.091	-0.879
18	0.325	0.333	0.389	-1.124	-1.100	-0.944
19	0.341	0.345	0.387	-1.076	-1.064	-0.949
20	0.305	0.351	0.365	-1.187	-1.047	-1.008

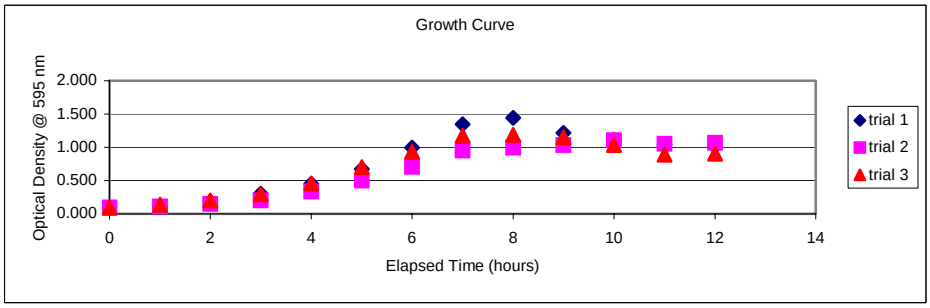


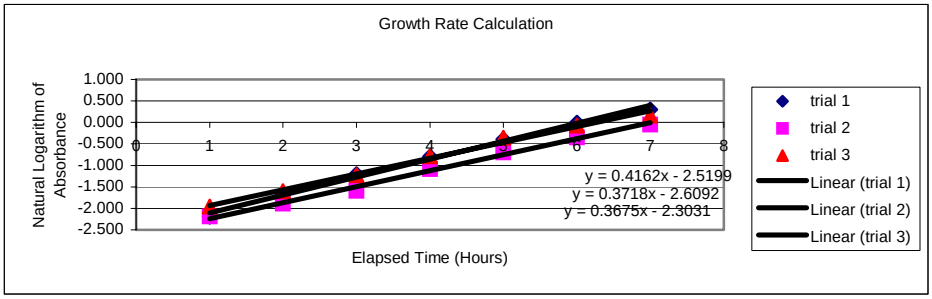


	Growth Rate			
trial 1	trial 2	trial 3	Average	STD
0.2020	0.1528	0.1788	0.1779	0.0246

pH 9

				Logarithms		
Elapsed Time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.092	0.095	0.090	-2.386	-2.354	-2.408
1	0.111	0.112	0.141	-2.198	-2.189	-1.959
2	0.185	0.151	0.202	-1.687	-1.890	-1.599
3	0.301	0.203	0.294	-1.201	-1.595	-1.224
4	0.451	0.337	0.455	-0.796	-1.088	-0.787
5	0.674	0.498	0.702	-0.395	-0.697	-0.354
6	0.994	0.707	0.933	-0.006	-0.347	-0.069
7	1.345	0.954	1.174	0.296	-0.047	0.160
8	1.440	0.994	1.187	0.365	-0.006	0.171
9	1.215	1.036	1.149	0.194	0.035	0.139
10	1.112	1.107	1.034	0.106	0.102	0.033
11	1.045	1.054	0.888	0.044	0.053	-0.119
12	1.014	1.065	0.902	0.014	0.063	-0.103

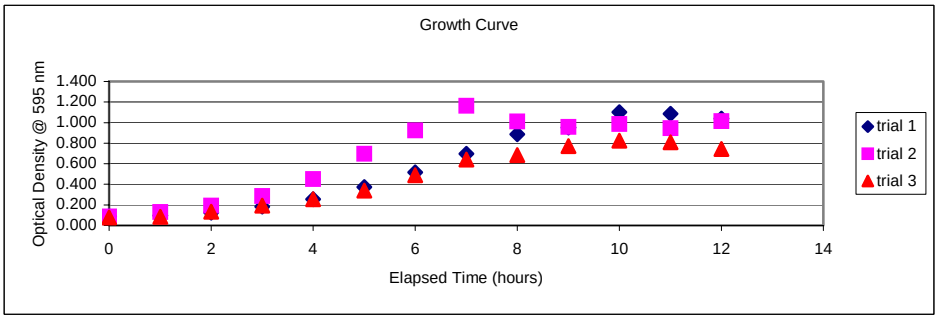


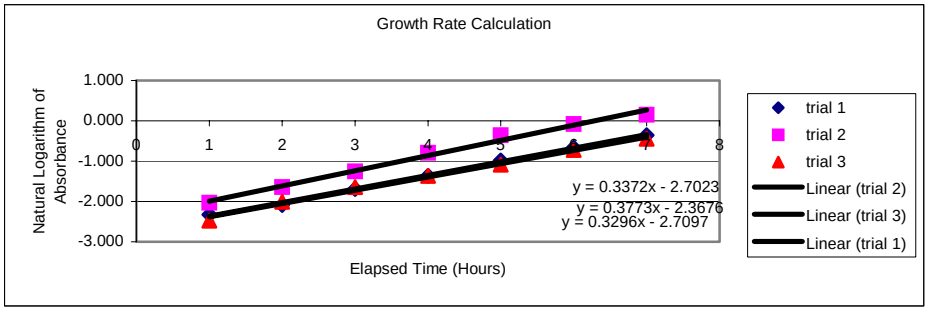


	Growth Rate			
trial 1	trial 2	trial 3	Average	STD
0.4162	0.3718	0.3675	0.3852	0.0270

pH 9.5

				Logarithms		
Elapsed Time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.089	0.088	0.077	-2.419	-2.430	-2.564
1	0.098	0.131	0.084	-2.323	-2.033	-2.477
2	0.124	0.194	0.134	-2.087	-1.640	-2.010
3	0.184	0.286	0.194	-1.693	-1.252	-1.640
4	0.256	0.451	0.256	-1.363	-0.796	-1.363
5	0.374	0.697	0.338	-0.983	-0.361	-1.085
6	0.515	0.923	0.487	-0.664	-0.080	-0.719
7	0.697	1.164	0.640	-0.361	0.152	-0.446
8	0.886	1.011	0.684	-0.121	0.011	-0.380
9	0.951	0.959	0.771	-0.050	-0.042	-0.260
10	1.102	0.987	0.824	0.097	-0.013	-0.194
11	1.085	0.945	0.808	0.082	-0.057	-0.213
12	1.040	1.014	0.745	0.039	0.014	-0.294

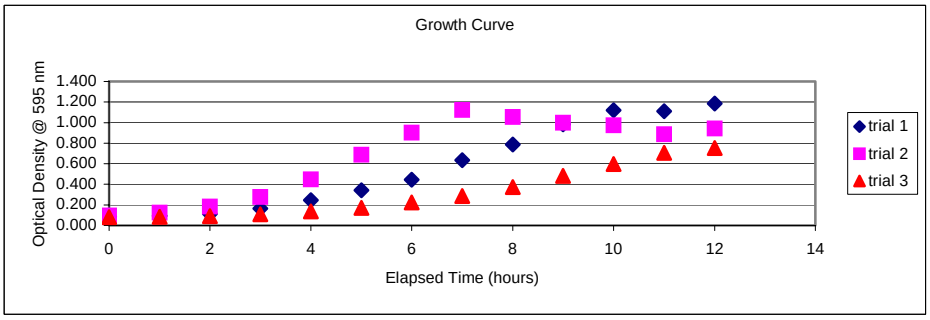


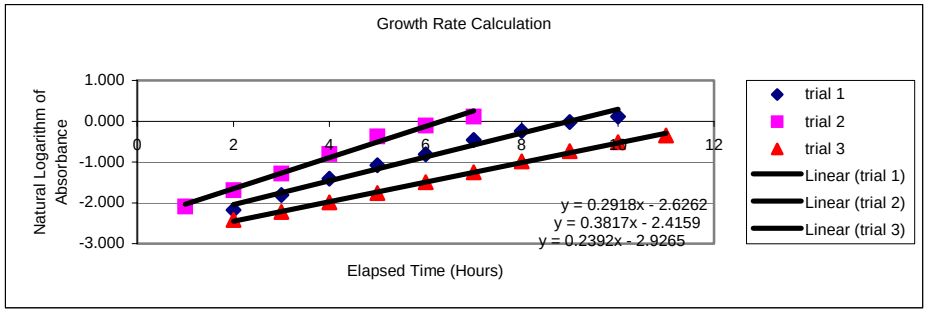


	Growth Rate			
trial 1	trial 2	trial 3	Average	STD
0.3372	0.3773	0.3296	0.3480	0.0256

pH 10

				Logarithms		
Elapsed Time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.086	0.098	0.081	-2.453	-2.323	-2.513
1	0.094	0.124	0.084	-2.364	-2.087	-2.477
2	0.113	0.185	0.089	-2.180	-1.687	-2.419
3	0.164	0.277	0.108	-1.808	-1.284	-2.226
4	0.245	0.448	0.137	-1.406	-0.803	-1.988
5	0.341	0.687	0.172	-1.076	-0.375	-1.760
6	0.445	0.902	0.224	-0.810	-0.103	-1.496
7	0.634	1.123	0.286	-0.456	0.116	-1.252
8	0.788	1.054	0.374	-0.238	0.053	-0.983
9	0.984	0.998	0.481	-0.016	-0.002	-0.732
10	1.121	0.975	0.597	0.114	-0.025	-0.516
11	1.112	0.887	0.706	0.106	-0.120	-0.348
12	1.185	0.942	0.754	0.170	-0.060	-0.282

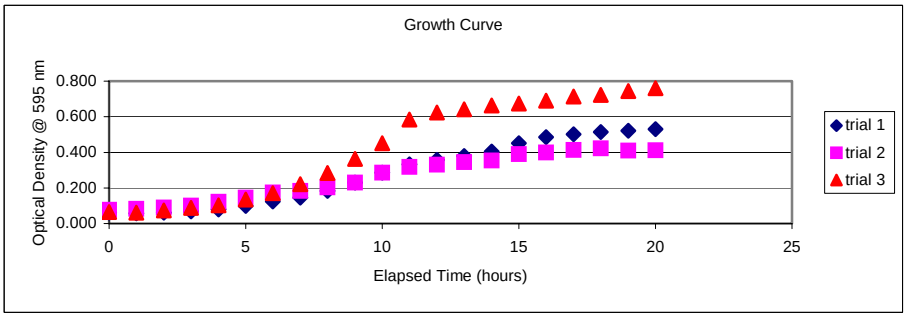


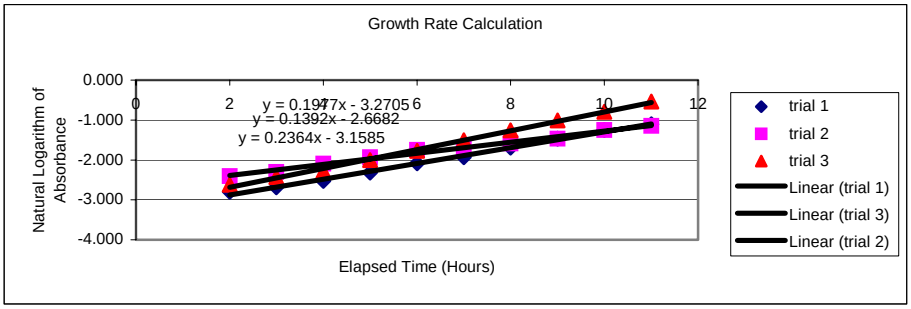


	Growth Rate			
trial 1	trial 2	trial 3	Average	STD
0.2918	0.3817	0.2392	0.3042	0.0721

pH 10.5

				Logarithms		
Elapsed Time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.061	0.078	0.065	-2.797	-2.551	-2.733
1	0.058	0.082	0.061	-2.847	-2.501	-2.797
2	0.061	0.090	0.073	-2.797	-2.408	-2.617
3	0.068	0.100	0.088	-2.688	-2.303	-2.430
4	0.080	0.123	0.102	-2.526	-2.096	-2.283
5	0.099	0.145	0.135	-2.313	-1.931	-2.002
6	0.124	0.174	0.170	-2.087	-1.749	-1.772
7	0.145	0.183	0.221	-1.931	-1.698	-1.510
8	0.184	0.204	0.284	-1.693	-1.590	-1.259
9	0.231	0.231	0.364	-1.465	-1.465	-1.011
10	0.286	0.286	0.451	-1.252	-1.252	-0.796
11	0.332	0.319	0.584	-1.103	-1.143	-0.538
12	0.358	0.331	0.624	-1.027	-1.106	-0.472
13	0.379	0.345	0.641	-0.970	-1.064	-0.445
14	0.404	0.355	0.664	-0.906	-1.036	-0.409
15	0.451	0.391	0.674	-0.796	-0.939	-0.395
16	0.485	0.399	0.691	-0.724	-0.919	-0.370
17	0.501	0.414	0.714	-0.691	-0.882	-0.337
18	0.514	0.422	0.723	-0.666	-0.863	-0.324
19	0.522	0.410	0.745	-0.650	-0.892	-0.294
20	0.530	0.412	0.761	-0.635	-0.887	-0.273

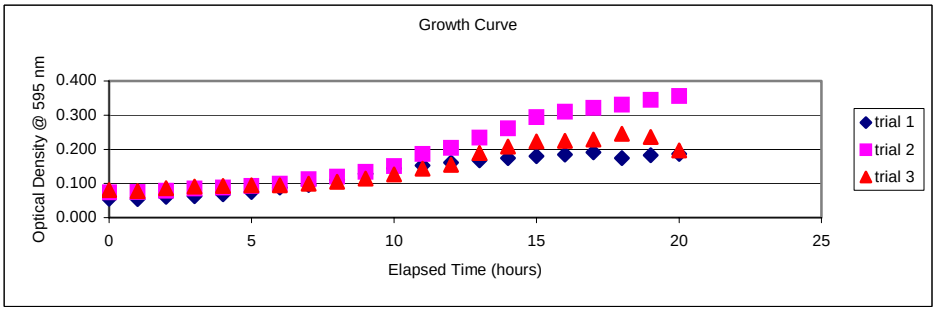


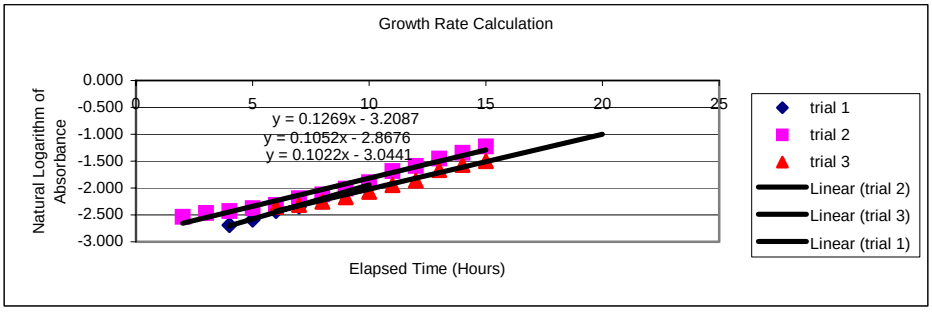


	Growth Rate			
trial 1	trial 2	trial 3	Average	STD
0.1977	0.1392	0.2364	0.1911	0.0489

pH 11

				Logarithms		
Elapsed Time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.055	0.074	0.081	-2.900	-2.604	-2.513
1	0.054	0.075	0.078	-2.919	-2.590	-2.551
2	0.061	0.079	0.086	-2.797	-2.538	-2.453
3	0.063	0.085	0.091	-2.765	-2.465	-2.397
4	0.068	0.088	0.093	-2.688	-2.430	-2.375
5	0.075	0.093	0.095	-2.590	-2.375	-2.354
6	0.088	0.099	0.095	-2.430	-2.313	-2.354
7	0.096	0.112	0.099	-2.343	-2.189	-2.313
8	0.111	0.120	0.105	-2.198	-2.120	-2.254
9	0.128	0.134	0.114	-2.056	-2.010	-2.172
10	0.144	0.151	0.126	-1.938	-1.890	-2.071
11	0.153	0.186	0.143	-1.877	-1.682	-1.945
12	0.161	0.204	0.155	-1.826	-1.590	-1.864
13	0.168	0.234	0.189	-1.784	-1.452	-1.666
14	0.174	0.261	0.208	-1.749	-1.343	-1.570
15	0.180	0.294	0.223	-1.715	-1.224	-1.501
16	0.185	0.310	0.225	-1.687	-1.171	-1.492
17	0.191	0.321	0.229	-1.655	-1.136	-1.474
18	0.174	0.331	0.245	-1.749	-1.106	-1.406
19	0.183	0.345	0.236	-1.698	-1.064	-1.444
20	0.186	0.356	0.197	-1.682	-1.033	-1.625

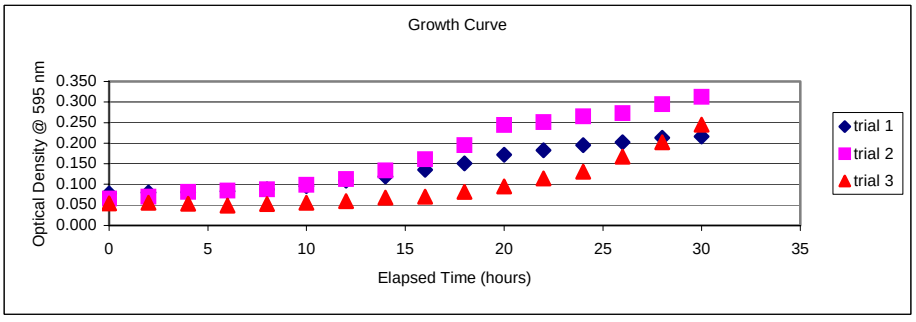


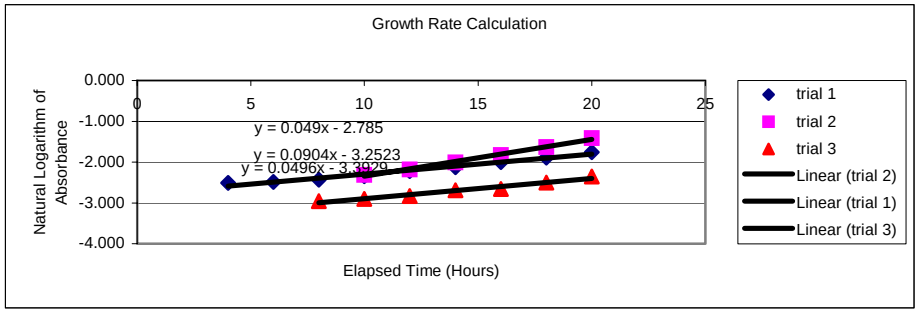


	Growth Rate			
trial 1	trial 2	trial 3	Average	STD
0.1269	0.1052	0.1022	0.1114	0.0135

pH 12

				Logarithms		
Elapsed Time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.078	0.065	0.054	-2.551	-2.733	-2.919
2	0.080	0.070	0.055	-2.526	-2.659	-2.900
4	0.081	0.082	0.053	-2.513	-2.501	-2.937
6	0.083	0.085	0.048	-2.489	-2.465	-3.037
8	0.088	0.088	0.052	-2.430	-2.430	-2.957
10	0.096	0.099	0.055	-2.343	-2.313	-2.900
12	0.109	0.113	0.059	-2.216	-2.180	-2.830
14	0.119	0.134	0.068	-2.129	-2.010	-2.688
16	0.135	0.161	0.070	-2.002	-1.826	-2.659
18	0.151	0.195	0.082	-1.890	-1.635	-2.501
20	0.172	0.244	0.095	-1.760	-1.411	-2.354
22	0.183	0.251	0.114	-1.698	-1.382	-2.172
24	0.195	0.265	0.131	-1.635	-1.328	-2.033
26	0.202	0.273	0.167	-1.599	-1.298	-1.790
28	0.213	0.295	0.202	-1.546	-1.221	-1.599
30	0.216	0.313	0.245	-1.532	-1.162	-1.406

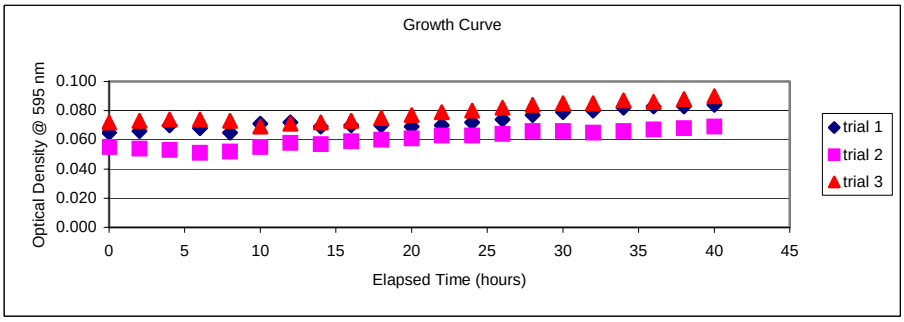


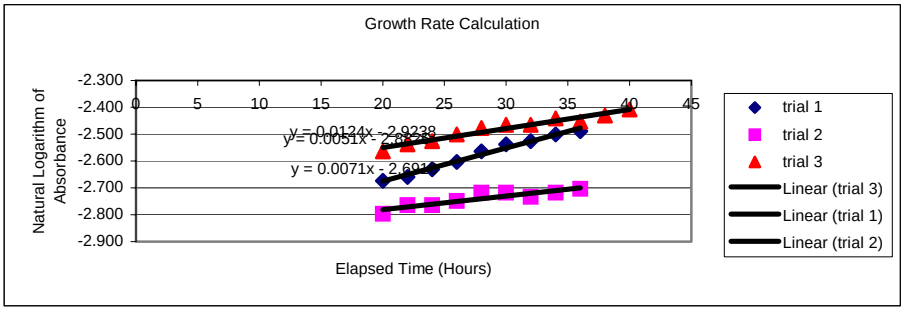


	Growth Rate			
trial 1	trial 2	trial 3	Average	STD
0.0490	0.0904	0.0496	0.0630	0.0237

pH 13

				Logarithms		
Elapsed Time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.065	0.055	0.072	-2.733	-2.900	-2.631
2	0.066	0.054	0.073	-2.718	-2.919	-2.617
4	0.070	0.053	0.074	-2.659	-2.937	-2.604
6	0.068	0.051	0.074	-2.688	-2.976	-2.604
8	0.065	0.052	0.073	-2.733	-2.957	-2.617
10	0.071	0.055	0.069	-2.645	-2.900	-2.674
12	0.072	0.058	0.071	-2.631	-2.847	-2.645
14	0.069	0.057	0.072	-2.674	-2.865	-2.631
16	0.070	0.059	0.073	-2.659	-2.830	-2.617
18	0.070	0.060	0.075	-2.659	-2.813	-2.590
20	0.069	0.061	0.077	-2.674	-2.797	-2.564
22	0.070	0.063	0.079	-2.659	-2.765	-2.538
24	0.072	0.063	0.080	-2.631	-2.765	-2.526
26	0.074	0.064	0.082	-2.604	-2.749	-2.501
28	0.077	0.066	0.084	-2.564	-2.718	-2.477
30	0.079	0.066	0.085	-2.538	-2.718	-2.465
32	0.080	0.065	0.085	-2.526	-2.733	-2.465
34	0.082	0.066	0.087	-2.501	-2.718	-2.442
36	0.083	0.067	0.086	-2.489	-2.703	-2.453
38	0.083	0.068	0.088	-2.489	-2.688	-2.430
40	0.084	0.069	0.090	-2.477	-2.674	-2.408

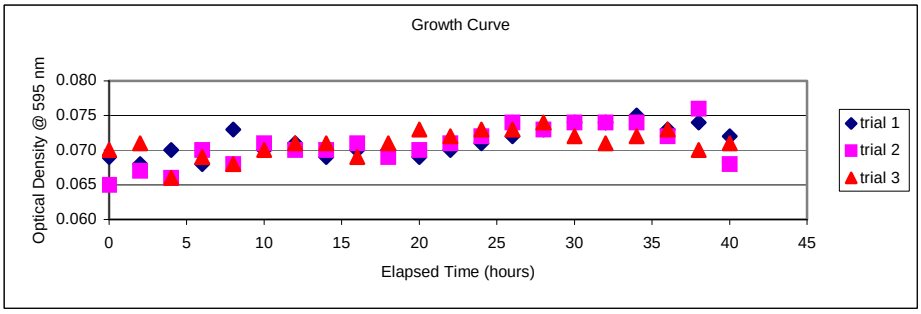


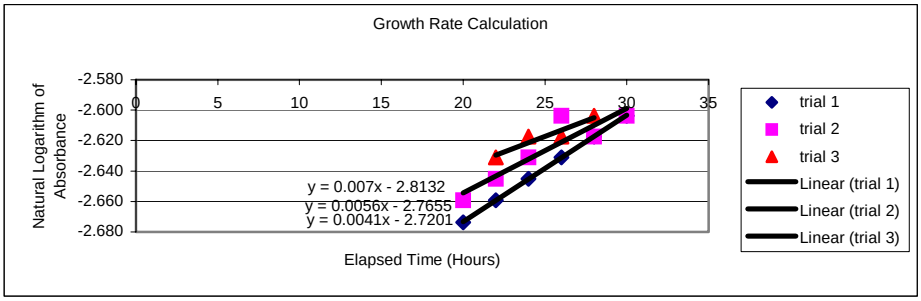


	Growth Rate			
trial 1	trial 2	trial 3	Average	STD
0.0124	0.0051	0.0071	0.0082	0.0038

pH 14

				Logarithms		
Elapsed Time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.069	0.065	0.070	-2.674	-2.733	-2.659
2	0.068	0.067	0.071	-2.688	-2.703	-2.645
4	0.070	0.066	0.066	-2.659	-2.718	-2.718
6	0.068	0.070	0.069	-2.688	-2.659	-2.674
8	0.073	0.068	0.068	-2.617	-2.688	-2.688
10	0.070	0.071	0.070	-2.659	-2.645	-2.659
12	0.071	0.070	0.071	-2.645	-2.659	-2.645
14	0.069	0.070	0.071	-2.674	-2.659	-2.645
16	0.070	0.071	0.069	-2.659	-2.645	-2.674
18	0.070	0.069	0.071	-2.659	-2.674	-2.645
20	0.069	0.070	0.073	-2.674	-2.659	-2.617
22	0.070	0.071	0.072	-2.659	-2.645	-2.631
24	0.071	0.072	0.073	-2.645	-2.631	-2.617
26	0.072	0.074	0.073	-2.631	-2.604	-2.617
28	0.073	0.073	0.074	-2.617	-2.617	-2.604
30	0.074	0.074	0.072	-2.604	-2.604	-2.631
32	0.074	0.074	0.071	-2.604	-2.604	-2.645
34	0.075	0.074	0.072	-2.590	-2.604	-2.631
36	0.073	0.072	0.073	-2.617	-2.631	-2.617
38	0.074	0.076	0.070	-2.604	-2.577	-2.659
40	0.072	0.068	0.071	-2.631	-2.688	-2.645





	Growth Rate			
trial 1	trial 2	trial 3	Average	STD
0.0070	0.0056	0.0041	0.0056	0.0015

Appendix D

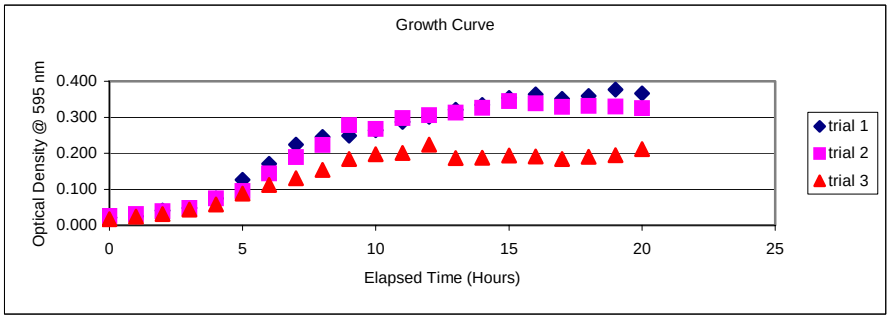
Appendix D presents optical density data gathered for the aerobic growth of *H. campisalis* over a wide range of salinities. All experiments were performed at pH 9, as this was the pH at which growth optima for *H. campisalis* occurred. The maximum specific growth rate was determined by plotting the natural logarithms of the optical density data versus elapsed growth time. A linear line was then fit to the steepest part of this data set. The slope of this line represented the maximum specific growth rate.

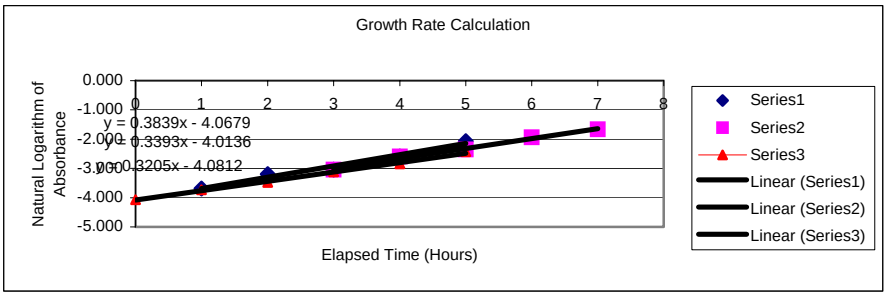
Growth kinetics for *H. campisalis* with varying salinity (Aerobic Conditions)

NaCl concentration (g/L)	Growth Rate h-1	Standard Derivations
0	0.3479	0.0326
5	0.3952	0.0273
10	0.4445	0.0371
20	0.5319	0.0436
30	0.3955	0.0332
60	0.3488	0.0252
90	0.3344	0.0332
115	0.2669	0.0143
145	0.1815	0.0185
175	0.1010	0.0104
205	0.0097	0.0025
235	0.0000	0.0000
260	0.0000	0.0000

0 grams/liter

					Logarithms	
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.021	0.026	0.017	-3.863	-3.650	-4.075
1	0.025	0.031	0.024	-3.689	-3.474	-3.730
2	0.041	0.039	0.031	-3.194	-3.244	-3.474
3	0.048	0.048	0.044	-3.037	-3.037	-3.124
4	0.075	0.075	0.058	-2.590	-2.590	-2.847
5	0.126	0.095	0.088	-2.071	-2.354	-2.430
6	0.171	0.144	0.112	-1.766	-1.938	-2.189
7	0.224	0.189	0.131	-1.496	-1.666	-2.033
8	0.245	0.223	0.154	-1.406	-1.501	-1.871
9	0.249	0.278	0.184	-1.390	-1.280	-1.693
10	0.264	0.268	0.197	-1.332	-1.317	-1.625
11	0.287	0.298	0.201	-1.248	-1.211	-1.604
12	0.301	0.306	0.224	-1.201	-1.184	-1.496
13	0.321	0.313	0.187	-1.136	-1.162	-1.677
14	0.334	0.326	0.188	-1.097	-1.121	-1.671
15	0.354	0.345	0.194	-1.038	-1.064	-1.640
16	0.364	0.339	0.191	-1.011	-1.082	-1.655
17	0.351	0.329	0.184	-1.047	-1.112	-1.693
18	0.359	0.332	0.190	-1.024	-1.103	-1.661
19	0.377	0.330	0.195	-0.976	-1.109	-1.635
20	0.366	0.325	0.212	-1.005	-1.124	-1.551

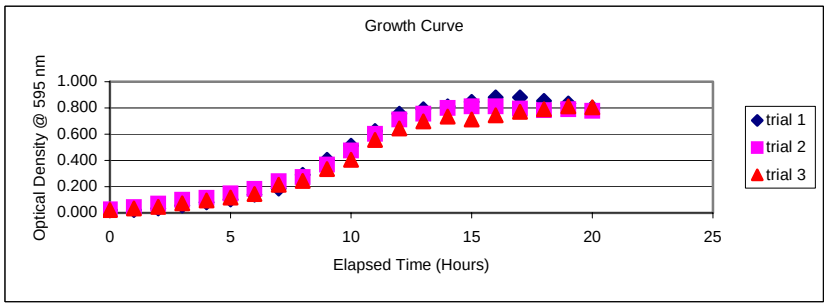


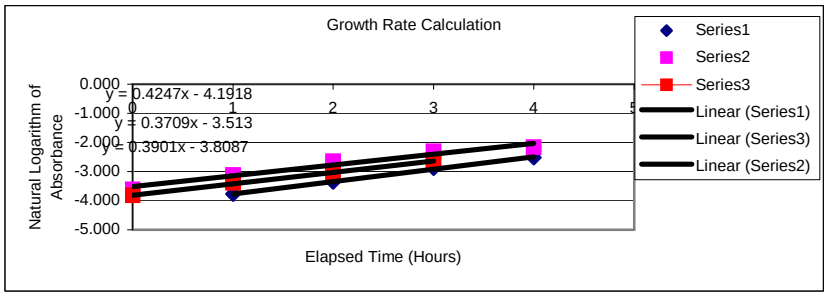


trial 1	trial 2	trial 3	Growth Rate	STD
0.3205	0.3393	0.3839	0.3479	0.0326

5 grams/liter

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.022	0.027	0.022	-3.817	-3.612	-3.817
1	0.023	0.044	0.034	-3.772	-3.124	-3.381
2	0.035	0.071	0.046	-3.352	-2.645	-3.079
3	0.056	0.099	0.073	-2.882	-2.313	-2.617
4	0.081	0.115	0.094	-2.513	-2.163	-2.364
5	0.102	0.149	0.116	-2.283	-1.904	-2.154
6	0.139	0.182	0.145	-1.973	-1.704	-1.931
7	0.185	0.241	0.215	-1.687	-1.423	-1.537
8	0.290	0.274	0.245	-1.238	-1.295	-1.406
9	0.406	0.368	0.334	-0.901	-1.000	-1.097
10	0.512	0.475	0.406	-0.669	-0.744	-0.901
11	0.624	0.603	0.557	-0.472	-0.506	-0.585
12	0.755	0.712	0.645	-0.281	-0.340	-0.439
13	0.791	0.755	0.697	-0.234	-0.281	-0.361
14	0.812	0.800	0.733	-0.208	-0.223	-0.311
15	0.846	0.812	0.712	-0.167	-0.208	-0.340
16	0.881	0.811	0.745	-0.127	-0.209	-0.294
17	0.880	0.795	0.771	-0.128	-0.229	-0.260
18	0.854	0.784	0.790	-0.158	-0.243	-0.236
19	0.833	0.791	0.812	-0.183	-0.234	-0.208
20	0.801	0.777	0.806	-0.222	-0.252	-0.216

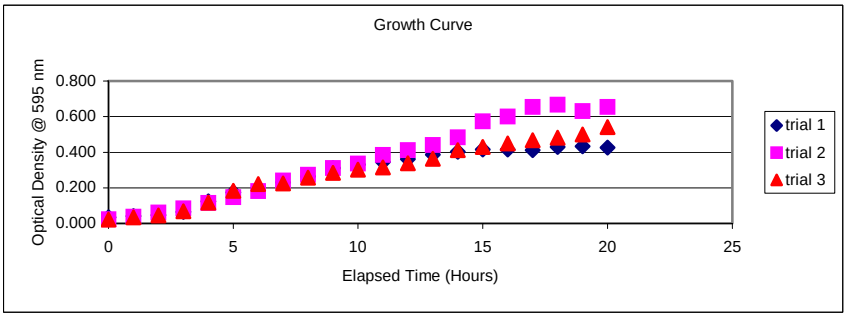


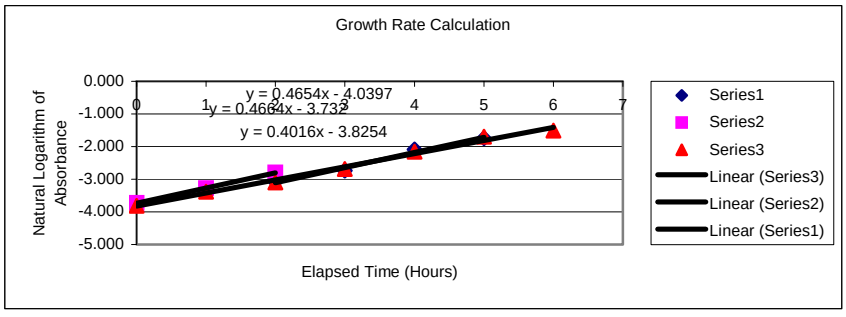


trial 1	trial 2	trial 3	Growth Rate	STD
0.4247	0.3709	0.3901	0.3952	0.0273

10 grams/liter

					Logarithms	
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.034	0.024	0.022	-3.381	-3.730	-3.817
1	0.041	0.038	0.034	-3.194	-3.270	-3.381
2	0.046	0.061	0.045	-3.079	-2.797	-3.101
3	0.065	0.084	0.068	-2.733	-2.477	-2.688
4	0.124	0.115	0.116	-2.087	-2.163	-2.154
5	0.175	0.147	0.184	-1.743	-1.917	-1.693
6	0.199	0.182	0.221	-1.614	-1.704	-1.510
7	0.231	0.241	0.224	-1.465	-1.423	-1.496
8	0.264	0.274	0.257	-1.332	-1.295	-1.359
9	0.311	0.311	0.284	-1.168	-1.168	-1.259
10	0.331	0.337	0.302	-1.106	-1.088	-1.197
11	0.345	0.385	0.315	-1.064	-0.955	-1.155
12	0.361	0.412	0.338	-1.019	-0.887	-1.085
13	0.387	0.441	0.364	-0.949	-0.819	-1.011
14	0.402	0.484	0.412	-0.911	-0.726	-0.887
15	0.415	0.574	0.429	-0.879	-0.555	-0.846
16	0.416	0.601	0.450	-0.877	-0.509	-0.799
17	0.412	0.654	0.467	-0.887	-0.425	-0.761
18	0.430	0.667	0.481	-0.844	-0.405	-0.732
19	0.433	0.631	0.499	-0.837	-0.460	-0.695
20	0.426	0.654	0.541	-0.853	-0.425	-0.614

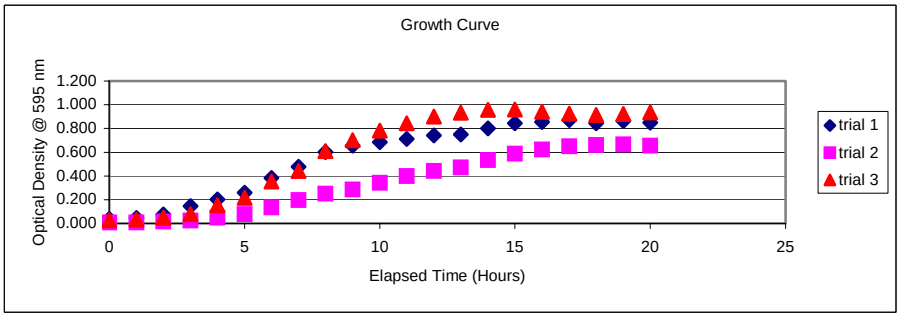


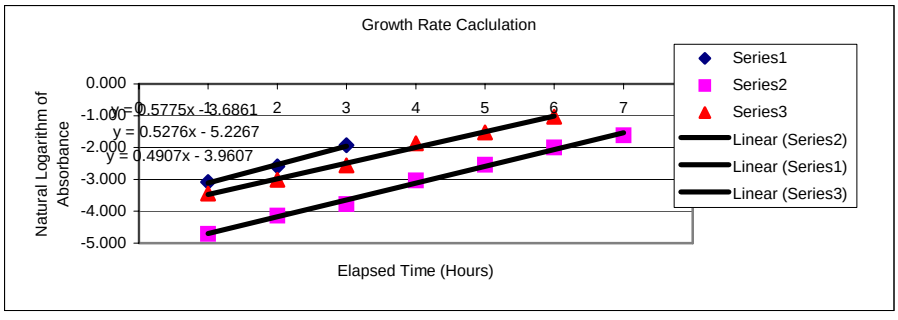


trial 1	trial 2	trial 3	Growth Rate	STD
0.4654	0.4664	0.4016	0.4445	0.0371

20 grams/liter

					Logarithms	
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.038	0.008	0.026	-3.270	-4.828	-3.650
1	0.046	0.009	0.032	-3.079	-4.711	-3.442
2	0.075	0.016	0.049	-2.590	-4.135	-3.016
3	0.146	0.023	0.077	-1.924	-3.772	-2.564
4	0.201	0.048	0.154	-1.604	-3.037	-1.871
5	0.259	0.079	0.217	-1.351	-2.538	-1.528
6	0.384	0.135	0.354	-0.957	-2.002	-1.038
7	0.477	0.198	0.441	-0.740	-1.619	-0.819
8	0.602	0.251	0.609	-0.507	-1.382	-0.496
9	0.655	0.287	0.700	-0.423	-1.248	-0.357
10	0.686	0.342	0.781	-0.377	-1.073	-0.247
11	0.713	0.398	0.845	-0.338	-0.921	-0.168
12	0.742	0.441	0.902	-0.298	-0.819	-0.103
13	0.751	0.471	0.932	-0.286	-0.753	-0.070
14	0.802	0.534	0.957	-0.221	-0.627	-0.044
15	0.845	0.589	0.960	-0.168	-0.529	-0.041
16	0.856	0.623	0.941	-0.155	-0.473	-0.061
17	0.870	0.651	0.924	-0.139	-0.429	-0.079
18	0.845	0.662	0.915	-0.168	-0.412	-0.089
19	0.864	0.665	0.922	-0.146	-0.408	-0.081
20	0.850	0.654	0.937	-0.163	-0.425	-0.065

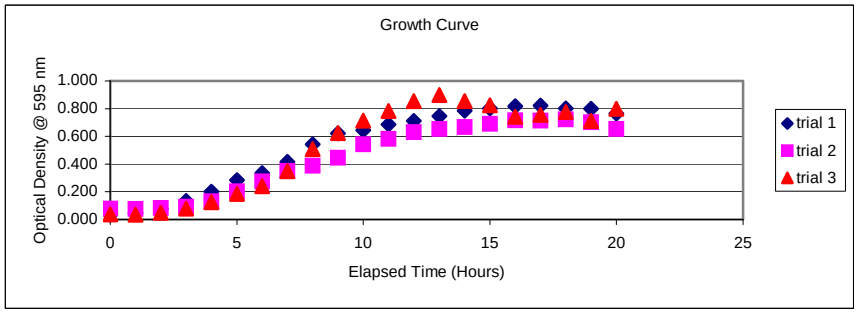


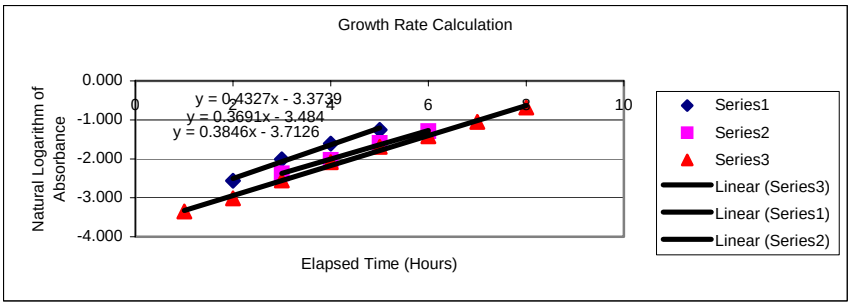


trial 1	trial 2	trial 3	Growth Rate	STD
0.5775	0.5276	0.4907	0.5319	0.0436

30 grams/liter

					Logarithms	
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.035	0.079	0.036	-3.352	-2.538	-3.324
1	0.046	0.077	0.035	-3.079	-2.564	-3.352
2	0.077	0.082	0.049	-2.564	-2.501	-3.016
3	0.134	0.093	0.078	-2.010	-2.375	-2.551
4	0.200	0.131	0.124	-1.609	-2.033	-2.087
5	0.285	0.203	0.184	-1.255	-1.595	-1.693
6	0.334	0.275	0.241	-1.097	-1.291	-1.423
7	0.415	0.350	0.349	-0.879	-1.050	-1.053
8	0.542	0.387	0.507	-0.612	-0.949	-0.679
9	0.621	0.446	0.624	-0.476	-0.807	-0.472
10	0.644	0.542	0.713	-0.440	-0.612	-0.338
11	0.687	0.583	0.784	-0.375	-0.540	-0.243
12	0.712	0.631	0.855	-0.340	-0.460	-0.157
13	0.745	0.654	0.899	-0.294	-0.425	-0.106
14	0.785	0.667	0.854	-0.242	-0.405	-0.158
15	0.802	0.690	0.824	-0.221	-0.371	-0.194
16	0.818	0.715	0.741	-0.201	-0.335	-0.300
17	0.823	0.714	0.756	-0.195	-0.337	-0.280
18	0.802	0.724	0.777	-0.221	-0.323	-0.252
19	0.799	0.702	0.712	-0.224	-0.354	-0.340
20	0.765	0.654	0.800	-0.268	-0.425	-0.223

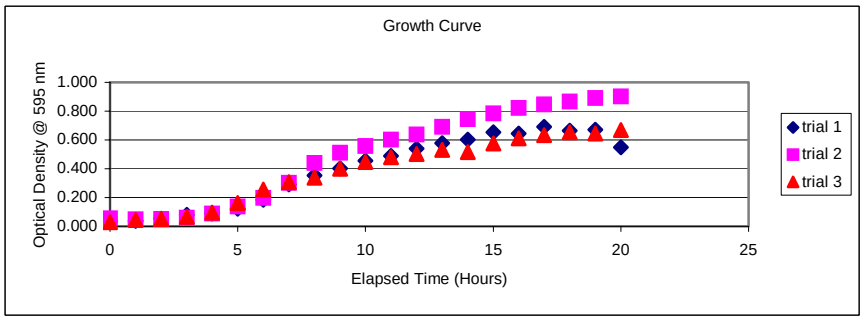


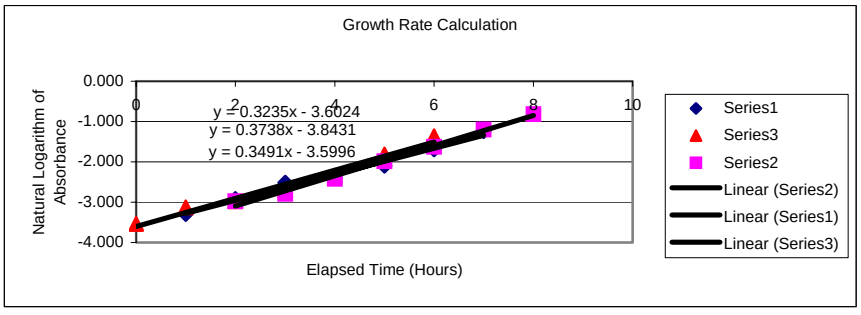


trial 1	trial 2	trial 3	Growth Rate	STD
0.4327	0.3691	0.3846	0.3955	0.0332

60 grams/liter

					Logarithms	
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.033	0.055	0.029	-3.411	-2.900	-3.540
1	0.037	0.049	0.044	-3.297	-3.016	-3.124
2	0.054	0.051	0.051	-2.919	-2.976	-2.976
3	0.081	0.061	0.067	-2.513	-2.797	-2.703
4	0.090	0.089	0.096	-2.408	-2.419	-2.343
5	0.123	0.137	0.163	-2.096	-1.988	-1.814
6	0.184	0.197	0.255	-1.693	-1.625	-1.366
7	0.291	0.302	0.306	-1.234	-1.197	-1.184
8	0.354	0.441	0.337	-1.038	-0.819	-1.088
9	0.402	0.512	0.399	-0.911	-0.669	-0.919
10	0.455	0.557	0.446	-0.787	-0.585	-0.807
11	0.490	0.602	0.481	-0.713	-0.507	-0.732
12	0.541	0.637	0.502	-0.614	-0.451	-0.689
13	0.577	0.691	0.531	-0.550	-0.370	-0.633
14	0.602	0.745	0.515	-0.507	-0.294	-0.664
15	0.654	0.784	0.575	-0.425	-0.243	-0.553
16	0.645	0.822	0.612	-0.439	-0.196	-0.491
17	0.691	0.846	0.633	-0.370	-0.167	-0.457
18	0.664	0.867	0.654	-0.409	-0.143	-0.425
19	0.671	0.891	0.645	-0.399	-0.115	-0.439
20	0.548	0.902	0.668	-0.601	-0.103	-0.403

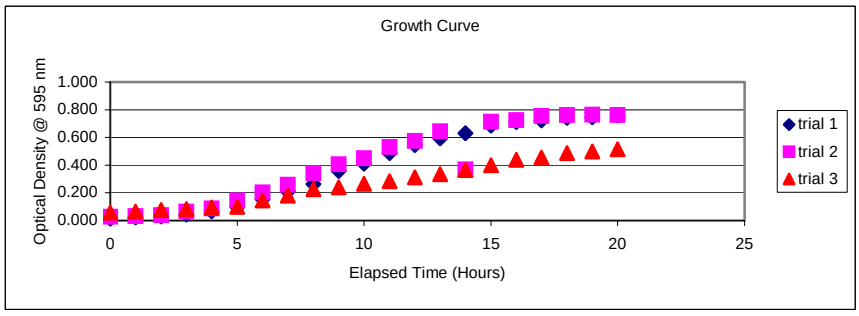


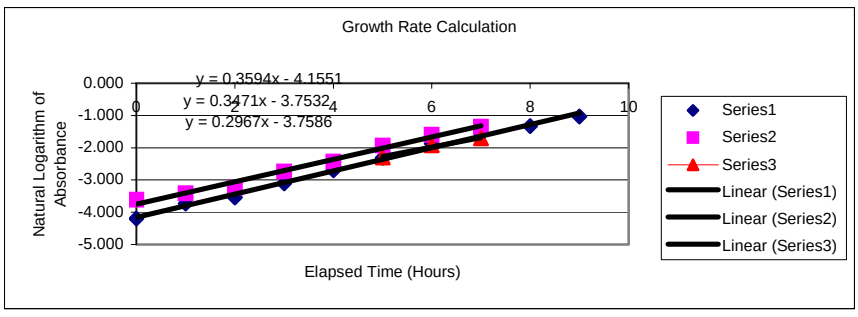


trial 1	trial 2	trial 3	Growth Rate	STD
0.3235	0.3738	0.3491	0.3488	0.0252

90 grams/liter

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.015	0.027	0.058	-4.200	-3.612	-2.847
1	0.024	0.033	0.064	-3.730	-3.411	-2.749
2	0.029	0.039	0.076	-3.540	-3.244	-2.577
3	0.045	0.065	0.082	-3.101	-2.733	-2.501
4	0.067	0.088	0.095	-2.703	-2.430	-2.354
5	0.099	0.145	0.100	-2.313	-1.931	-2.303
6	0.154	0.203	0.146	-1.871	-1.595	-1.924
7	0.210	0.259	0.181	-1.561	-1.351	-1.709
8	0.264	0.342	0.226	-1.332	-1.073	-1.487
9	0.357	0.406	0.241	-1.030	-0.901	-1.423
10	0.412	0.451	0.265	-0.887	-0.796	-1.328
11	0.487	0.531	0.284	-0.719	-0.633	-1.259
12	0.544	0.574	0.312	-0.609	-0.555	-1.165
13	0.594	0.645	0.334	-0.521	-0.439	-1.097
14	0.631	0.369	0.365	-0.460	-0.997	-1.008
15	0.686	0.713	0.400	-0.377	-0.338	-0.916
16	0.712	0.725	0.439	-0.340	-0.322	-0.823
17	0.724	0.756	0.455	-0.323	-0.280	-0.787
18	0.741	0.761	0.487	-0.300	-0.273	-0.719
19	0.745	0.765	0.499	-0.294	-0.268	-0.695
20	0.760	0.762	0.515	-0.274	-0.272	-0.664

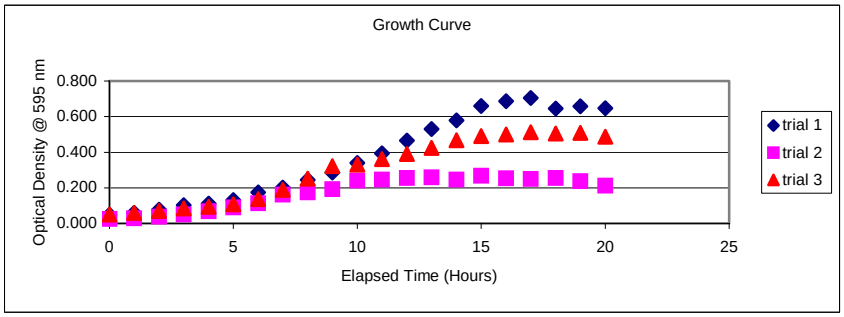


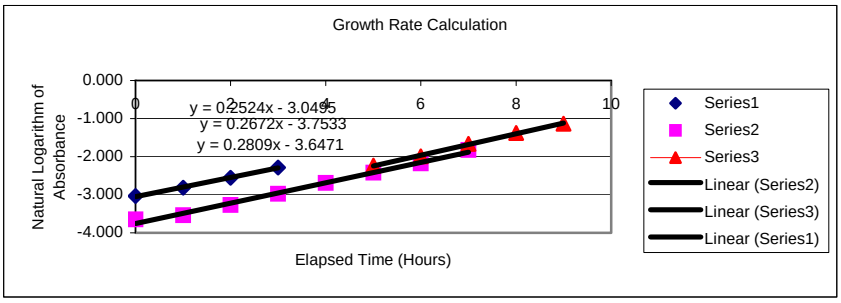


trial 1	trial 2	trial 3	Growth Rate	STD
0.3594	0.3471	0.2967	0.3344	0.0332

115 grams/liter

					Logarithms	
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.048	0.026	0.050	-3.037	-3.650	-2.996
1	0.060	0.029	0.060	-2.813	-3.540	-2.813
2	0.078	0.038	0.071	-2.551	-3.270	-2.645
3	0.102	0.051	0.085	-2.283	-2.976	-2.465
4	0.112	0.068	0.093	-2.189	-2.688	-2.375
5	0.131	0.089	0.107	-2.033	-2.419	-2.235
6	0.174	0.113	0.137	-1.749	-2.180	-1.988
7	0.202	0.162	0.189	-1.599	-1.820	-1.666
8	0.245	0.175	0.251	-1.406	-1.743	-1.382
9	0.286	0.192	0.322	-1.252	-1.650	-1.133
10	0.339	0.241	0.331	-1.082	-1.423	-1.106
11	0.394	0.246	0.362	-0.931	-1.402	-1.016
12	0.465	0.255	0.390	-0.766	-1.366	-0.942
13	0.530	0.259	0.425	-0.635	-1.351	-0.856
14	0.578	0.246	0.467	-0.548	-1.402	-0.761
15	0.659	0.267	0.490	-0.417	-1.321	-0.713
16	0.686	0.254	0.500	-0.377	-1.370	-0.693
17	0.705	0.249	0.512	-0.350	-1.390	-0.669
18	0.645	0.256	0.506	-0.439	-1.363	-0.681
19	0.658	0.237	0.509	-0.419	-1.440	-0.675
20	0.647	0.212	0.488	-0.435	-1.551	-0.717

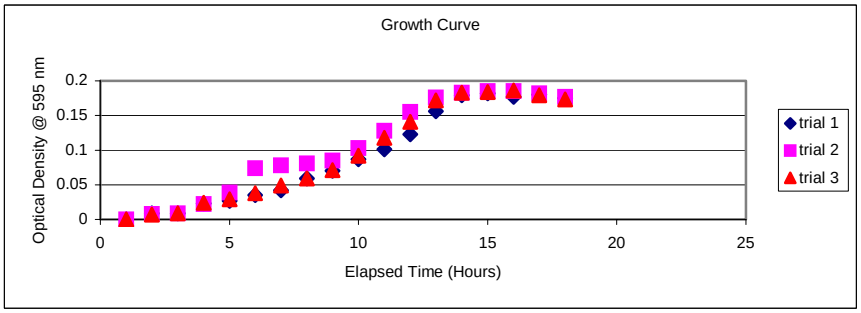


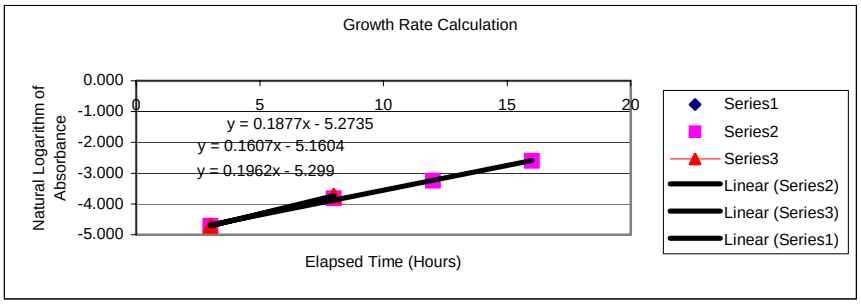


trial 1	trial 2	trial 3	Growth Rate	STD
0.2524	0.2672	0.2809	0.2668	0.0143

145 grams/liter

					Logarithms	
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.009	0.008	0.007	-4.711	-4.828	-4.962
3	0.009	0.009	0.009	-4.711	-4.711	-4.711
8	0.023	0.022	0.024	-3.772	-3.817	-3.730
12	0.027	0.039	0.029	-3.612	-3.244	-3.540
16	0.035	0.074	0.038	-3.352	-2.604	-3.270
20	0.042	0.078	0.049	-3.170	-2.551	-3.016
22	0.059	0.081	0.059	-2.830	-2.513	-2.830
23	0.070	0.085	0.071	-2.659	-2.465	-2.645
25	0.087	0.103	0.092	-2.442	-2.273	-2.386
27	0.101	0.128	0.118	-2.293	-2.056	-2.137
29	0.123	0.155	0.141	-2.096	-1.864	-1.959
30	0.156	0.176	0.172	-1.858	-1.737	-1.760
31	0.179	0.183	0.183	-1.720	-1.698	-1.698
32	0.182	0.185	0.184	-1.704	-1.687	-1.693
33	0.177	0.185	0.186	-1.732	-1.687	-1.682
34	0.180	0.182	0.179	-1.715	-1.704	-1.720
35	0.175	0.177	0.173	-1.743	-1.732	-1.754

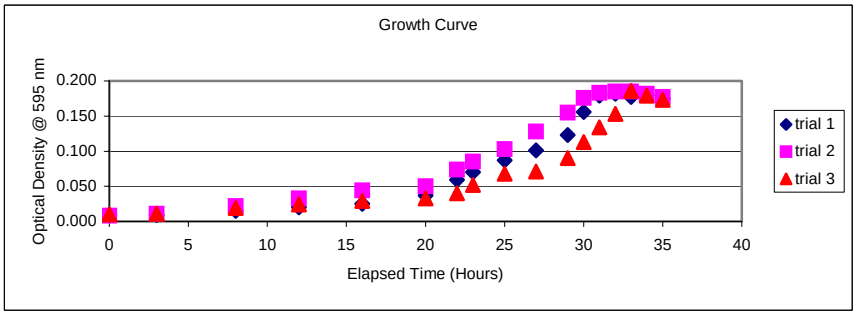


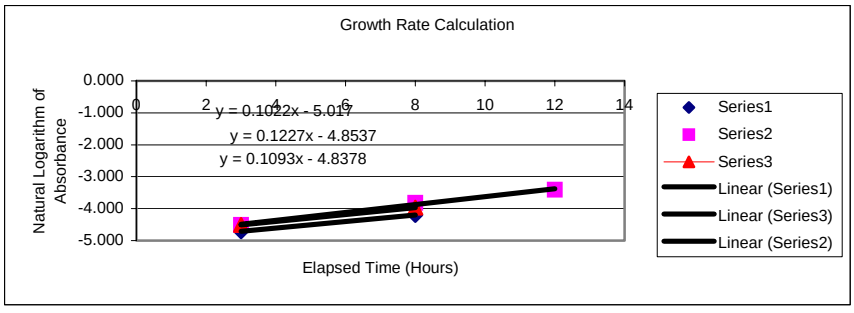


trial 1	trial 2	trial 3	Growth Rate	STD
0.1877	0.1607	0.1962	0.1815	0.0185

175 grams/liter

					Logarithms	
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.009	0.008	0.010	-4.711	-4.828	-4.605
3	0.009	0.011	0.011	-4.711	-4.510	-4.510
8	0.015	0.022	0.019	-4.200	-3.817	-3.963
12	0.020	0.033	0.024	-3.912	-3.411	-3.730
16	0.025	0.044	0.029	-3.689	-3.124	-3.540
20	0.037	0.050	0.033	-3.297	-2.996	-3.411
22	0.059	0.074	0.040	-2.830	-2.604	-3.219
23	0.070	0.085	0.052	-2.659	-2.465	-2.957
25	0.087	0.103	0.068	-2.442	-2.273	-2.688
27	0.101	0.128	0.071	-2.293	-2.056	-2.645
29	0.123	0.155	0.090	-2.096	-1.864	-2.408
30	0.156	0.176	0.113	-1.858	-1.737	-2.180
31	0.179	0.183	0.134	-1.720	-1.698	-2.010
32	0.182	0.185	0.153	-1.704	-1.687	-1.877
33	0.177	0.185	0.186	-1.732	-1.687	-1.682
34	0.180	0.182	0.179	-1.715	-1.704	-1.720
35	0.175	0.177	0.173	-1.743	-1.732	-1.754

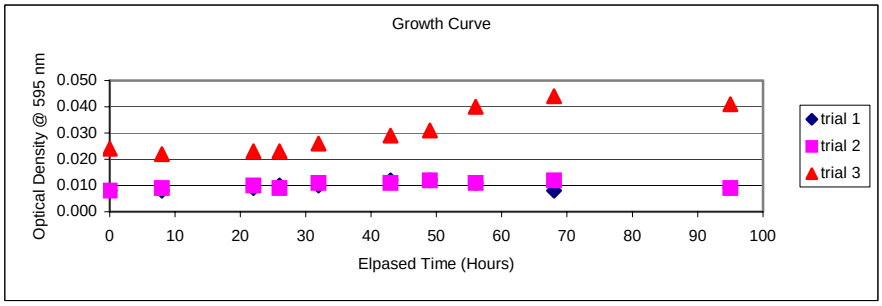


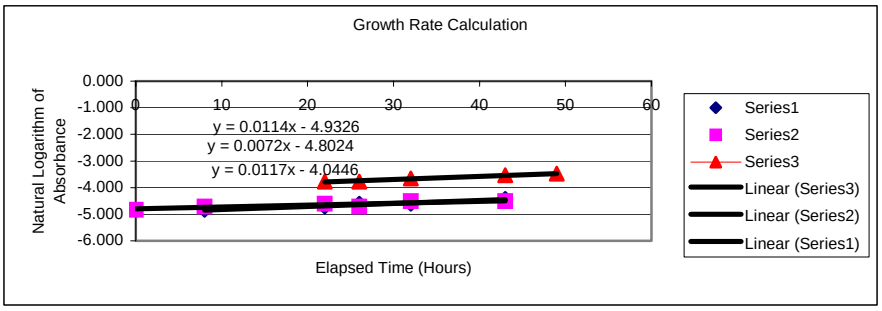


trial 1	trial 2	trial 3	Growth Rate	STD
0.1022	0.1227	0.1093	0.1114	0.0104

205 grams/liter

					Logarithms	
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.008	0.008	0.024	-4.828	-4.828	-3.730
8	0.008	0.009	0.022	-4.828	-4.711	-3.817
22	0.009	0.010	0.023	-4.711	-4.605	-3.772
26	0.010	0.009	0.023	-4.605	-4.711	-3.772
32	0.010	0.011	0.026	-4.605	-4.510	-3.650
43	0.012	0.011	0.029	-4.423	-4.510	-3.540
49	0.012	0.012	0.031	-4.423	-4.423	-3.474
56	0.011	0.011	0.040	-4.510	-4.510	-3.219
68	0.008	0.012	0.044	-4.828	-4.423	-3.124
95	0.009	0.009	0.041	-4.711	-4.711	-3.194

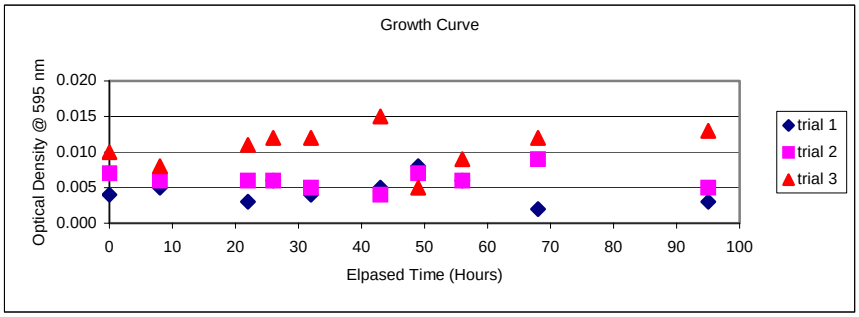




trial 1	trial 2	trial 3	Growth Rate	STD
0.0114	0.0072	0.0117	0.0101	0.0025

235 grams/liter

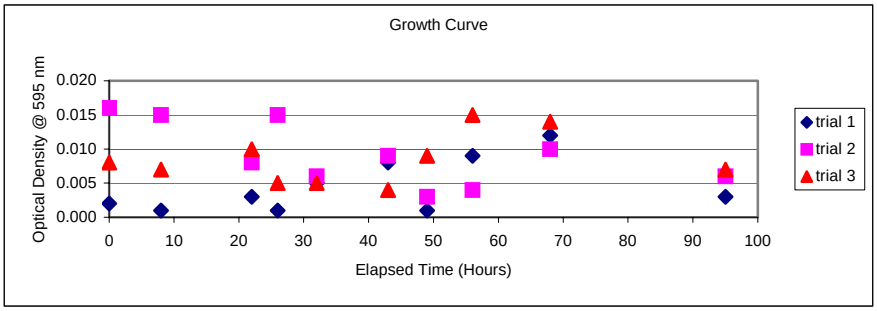
				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.004	0.007	0.010	-5.521	-4.962	-4.605
8	0.005	0.006	0.008	-5.298	-5.116	-4.828
22	0.003	0.006	0.011	-5.809	-5.116	-4.510
26	0.006	0.006	0.012	-5.116	-5.116	-4.423
32	0.004	0.005	0.012	-5.521	-5.298	-4.423
43	0.005	0.004	0.015	-5.298	-5.521	-4.200
49	0.008	0.007	0.005	-4.828	-4.962	-5.298
56	0.006	0.006	0.009	-5.116	-5.116	-4.711
68	0.002	0.009	0.012	-6.215	-4.711	-4.423
95	0.003	0.005	0.013	-5.809	-5.298	-4.343



trial 1	trial 2	trial 3	Growth Rate	STD
0.0000	0.0000	0.0000	0.0000	0.0000

260 grams/liter

					Logarithms	
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.002	0.016	0.008	-6.215	-4.135	-4.828
8	0.001	0.015	0.007	-6.908	-4.200	-4.962
22	0.003	0.008	0.010	-5.809	-4.828	-4.605
26	0.001	0.015	0.005	-6.908	-4.200	-5.298
32	0.005	0.006	0.005	-5.298	-5.116	-5.298
43	0.008	0.009	0.004	-4.828	-4.711	-5.521
49	0.001	0.003	0.009	-6.908	-5.809	-4.711
56	0.009	0.004	0.015	-4.711	-5.521	-4.200
68	0.012	0.010	0.014	-4.423	-4.605	-4.269
95	0.003	0.006	0.007	-5.809	-5.116	-4.962



trial 1	trial 2	trial 3	Growth Rate	STD
0.0000	0.0000	0.0000	0.0000	0.0000

Appendix E

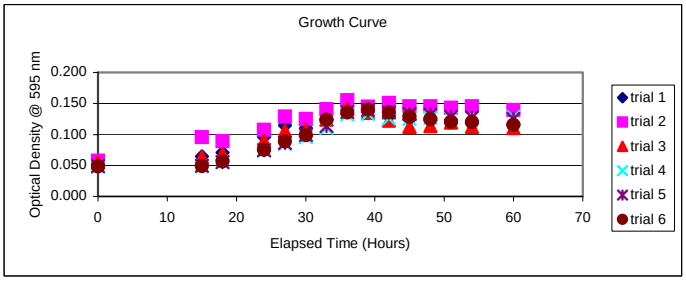
Appendix E presents optical density data gathered for the denitrifying growth of *H. campisalis* over a wide range of salinities. All experiments were performed at pH 9, as this was the pH at which growth optima for *H. campisalis* occurred. The maximum specific growth rate was determined by plotting the natural logarithms of the optical density data versus elapsed growth time. A linear line was then fit to the steepest part of this data set. The slope of this line represented the maximum specific growth rate.

Growth kinetics for *H. campisalis* with varying salinity (denitrifying conditions)

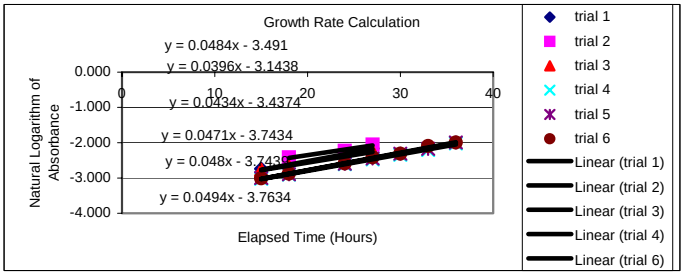
NaCl (g/L)	Growth Rate	STD
0	0.0460	0.0037
5	0.0824	0.0085
10	0.1326	0.0074
15	0.1254	0.0044
20	0.1374	0.0044
30	0.3321	0.0218
45	0.2971	0.0173
60	0.2842	0.0101
80	0.2666	0.0212
90	0.1470	0.0177
100	0.1719	0.0106
115	0.1081	0.0038
145	0.0153	0.0029
175	0.0094	0.0021
205	0.0074	0.0019
235	0.0000	0.0000
260	0.0000	0.0000

0 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.052	0.058	0.055	0.048	0.049	0.049	-2.949	-2.851	-2.904	-3.047	-3.014	-3.024
15	0.065	0.096	0.063	0.049	0.050	0.049	-2.741	-2.345	-2.763	-3.026	-3.004	-3.014
18	0.071	0.089	0.068	0.055	0.055	0.056	-2.648	-2.418	-2.682	-2.902	-2.895	-2.879
24	0.096	0.107	0.090	0.073	0.075	0.075	-2.344	-2.231	-2.410	-2.613	-2.596	-2.589
27	0.115	0.129	0.106	0.085	0.086	0.089	-2.167	-2.049	-2.248	-2.471	-2.449	-2.421
30	0.111	0.125	0.107	0.096	0.099	0.099	-2.203	-2.077	-2.240	-2.348	-2.309	-2.316
33	0.124	0.141	0.124	0.110	0.113	0.124	-2.087	-1.960	-2.087	-2.205	-2.177	-2.091
36	0.140	0.155	0.141	0.132	0.136	0.135	-1.963	-1.862	-1.957	-2.024	-1.992	-2.000
39	0.137	0.145	0.135	0.133	0.140	0.140	-1.988	-1.930	-2.005	-2.019	-1.968	-1.965
42	0.130	0.151	0.122	0.126	0.135	0.136	-2.044	-1.891	-2.100	-2.074	-2.000	-1.998
45	0.124	0.146	0.112	0.125	0.133	0.129	-2.087	-1.927	-2.187	-2.076	-2.017	-2.049
48	0.126	0.146	0.114	0.127	0.132	0.125	-2.075	-1.928	-2.173	-2.065	-2.024	-2.083
51	0.123	0.143	0.119	0.127	0.129	0.121	-2.092	-1.943	-2.128	-2.063	-2.051	-2.116
54	0.121	0.145	0.112	0.127	0.128	0.120	-2.111	-1.928	-2.186	-2.064	-2.060	-2.120
60	0.116	0.140	0.111	0.128	0.125	0.116	-2.156	-1.968	-2.203	-2.053	-2.079	-2.158

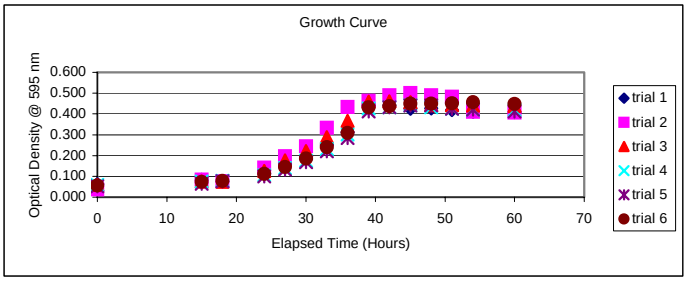


	Growth Rate
trial 1	0.0484
trial 2	0.0396
trial 3	0.0434
trial 4	0.0471
trial 5	0.0480
trial 6	0.0494
Average	0.0460
STD	0.0037

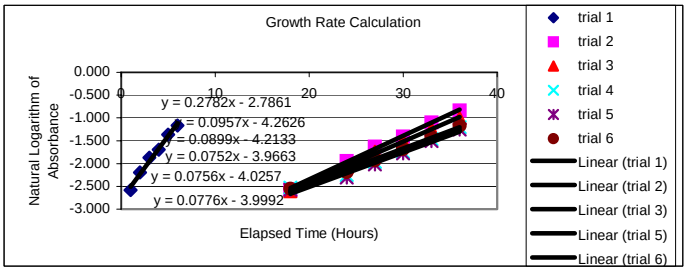


5 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.063	0.037	0.059	0.059	0.053	0.057	-2.771	-3.308	-2.832	-2.835	-2.945	-2.872
15	0.083	0.085	0.075	0.075	0.065	0.074	-2.490	-2.463	-2.597	-2.596	-2.736	-2.610
18	0.076	0.076	0.074	0.080	0.076	0.078	-2.581	-2.572	-2.609	-2.528	-2.578	-2.552
24	0.112	0.143	0.125	0.106	0.100	0.112	-2.193	-1.946	-2.076	-2.249	-2.308	-2.187
27	0.154	0.197	0.176	0.140	0.132	0.146	-1.872	-1.625	-1.739	-1.967	-2.023	-1.928
30	0.183	0.244	0.222	0.175	0.169	0.185	-1.698	-1.409	-1.505	-1.745	-1.780	-1.686
33	0.255	0.333	0.288	0.231	0.221	0.241	-1.368	-1.100	-1.245	-1.466	-1.512	-1.425
36	0.312	0.433	0.367	0.300	0.285	0.309	-1.164	-0.838	-1.003	-1.203	-1.256	-1.175
39	0.447	0.463	0.459	0.427	0.412	0.432	-0.806	-0.770	-0.779	-0.852	-0.888	-0.840
42	0.441	0.489	0.461	0.432	0.433	0.438	-0.818	-0.716	-0.774	-0.838	-0.838	-0.826
45	0.425	0.500	0.457	0.441	0.440	0.450	-0.857	-0.694	-0.783	-0.819	-0.821	-0.799
48	0.426	0.489	0.441	0.432	0.441	0.450	-0.853	-0.715	-0.818	-0.839	-0.818	-0.798
51	0.418	0.482	0.441	0.425	0.426	0.451	-0.872	-0.730	-0.819	-0.855	-0.854	-0.796
54	0.410	0.411	0.440	0.421	0.422	0.457	-0.891	-0.890	-0.821	-0.866	-0.864	-0.783
60	0.409	0.405	0.439	0.419	0.411	0.449	-0.895	-0.903	-0.824	-0.870	-0.890	-0.801

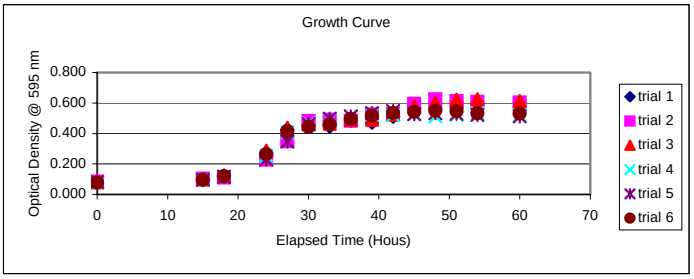


	Growth Rate
trial 1	0.0805
trial 2	0.0957
trial 3	0.0899
trial 4	0.0752
trial 5	0.0756
trial 6	0.0776
Average	0.0824
STD	0.0085

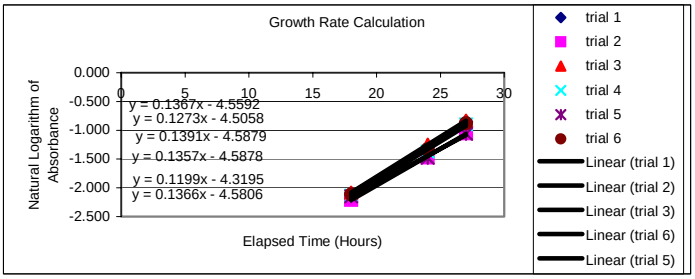


10 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.078	0.085	0.080	0.075	0.081	0.078	-2.550	-2.469	-2.528	-2.588	-2.511	-2.555
15	0.099	0.104	0.099	0.097	0.096	0.095	-2.314	-2.267	-2.316	-2.331	-2.339	-2.352
18	0.124	0.110	0.125	0.119	0.117	0.121	-2.092	-2.206	-2.083	-2.133	-2.150	-2.114
24	0.273	0.229	0.286	0.255	0.228	0.265	-1.299	-1.474	-1.250	-1.368	-1.477	-1.328
27	0.426	0.349	0.436	0.407	0.347	0.417	-0.854	-1.053	-0.831	-0.899	-1.059	-0.876
30	0.457	0.482	0.460	0.466	0.461	0.442	-0.782	-0.729	-0.776	-0.764	-0.775	-0.817
33	0.443	0.489	0.465	0.491	0.496	0.459	-0.814	-0.715	-0.765	-0.712	-0.700	-0.779
36	0.484	0.483	0.491	0.513	0.511	0.495	-0.726	-0.728	-0.711	-0.667	-0.672	-0.704
39	0.470	0.525	0.491	0.524	0.533	0.514	-0.756	-0.645	-0.711	-0.646	-0.629	-0.665
42	0.509	0.529	0.524	0.520	0.547	0.535	-0.675	-0.637	-0.646	-0.654	-0.604	-0.626
45	0.563	0.594	0.578	0.526	0.526	0.541	-0.574	-0.521	-0.549	-0.642	-0.642	-0.614
48	0.599	0.623	0.600	0.512	0.533	0.550	-0.513	-0.473	-0.510	-0.670	-0.629	-0.598
51	0.610	0.612	0.622	0.521	0.529	0.545	-0.494	-0.490	-0.476	-0.652	-0.637	-0.607
54	0.601	0.608	0.623	0.533	0.521	0.532	-0.509	-0.498	-0.473	-0.629	-0.652	-0.632
60	0.602	0.603	0.613	0.522	0.512	0.531	-0.507	-0.505	-0.490	-0.651	-0.670	-0.633

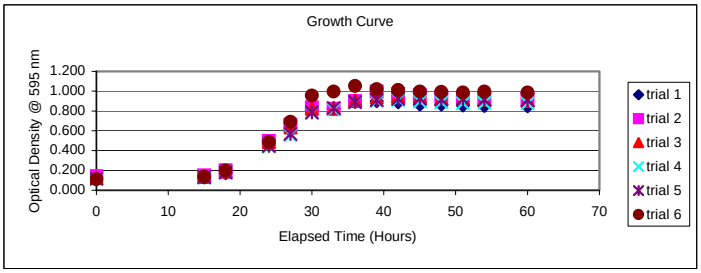


	Growth Rate
trial 1	0.1368
trial 2	0.1273
trial 3	0.1391
trial 4	0.1357
trial 5	0.1199
trial 6	0.1366
Average	0.1326
STD	0.0074

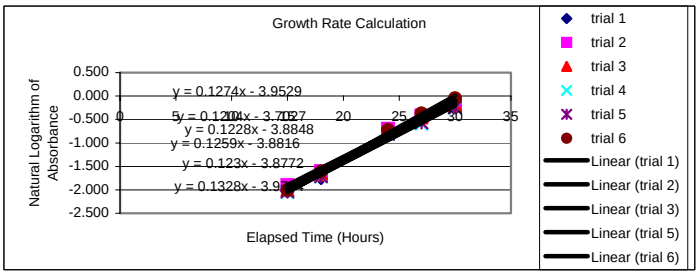


15 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.129	0.141	0.126	0.116	0.117	0.112	-2.048	-1.958	-2.075	-2.158	-2.146	-2.187
15	0.131	0.150	0.135	0.129	0.130	0.136	-2.030	-1.898	-2.006	-2.050	-2.039	-1.999
18	0.175	0.201	0.187	0.179	0.181	0.200	-1.746	-1.603	-1.675	-1.721	-1.712	-1.611
24	0.467	0.499	0.487	0.442	0.447	0.485	-0.761	-0.696	-0.719	-0.817	-0.806	-0.723
27	0.605	0.660	0.634	0.556	0.570	0.690	-0.502	-0.416	-0.456	-0.587	-0.562	-0.371
30	0.815	0.836	0.822	0.779	0.785	0.955	-0.204	-0.180	-0.197	-0.250	-0.243	-0.046
33	0.825	0.821	0.823	0.815	0.831	0.995	-0.193	-0.198	-0.194	-0.205	-0.185	-0.005
36	0.905	0.900	0.905	0.888	0.890	1.053	-0.100	-0.105	-0.099	-0.119	-0.116	0.052
39	0.897	0.994	0.937	0.902	0.910	1.021	-0.108	-0.006	-0.066	-0.103	-0.095	0.021
42	0.888	0.969	0.944	0.912	0.923	1.012	-0.119	-0.031	-0.058	-0.092	-0.080	0.012
45	0.865	0.958	0.943	0.895	0.927	0.996	-0.145	-0.043	-0.059	-0.111	-0.076	-0.004
48	0.864	0.945	0.923	0.888	0.916	0.992	-0.146	-0.057	-0.080	-0.119	-0.088	-0.009
51	0.857	0.933	0.925	0.880	0.923	0.988	-0.155	-0.069	-0.079	-0.128	-0.081	-0.013
54	0.851	0.922	0.912	0.880	0.915	0.996	-0.161	-0.082	-0.092	-0.128	-0.089	-0.004
60	0.848	0.920	0.901	0.879	0.912	0.987	-0.165	-0.083	-0.105	-0.129	-0.092	-0.013

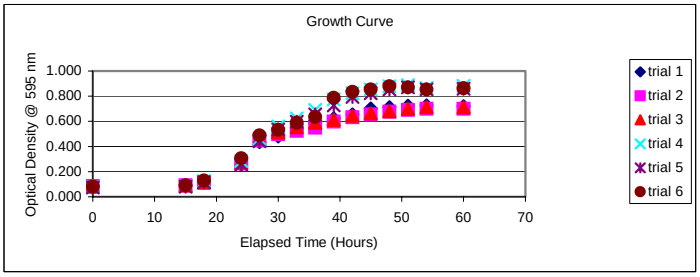


	Growth Rate
trial 1	0.1274
trial 2	0.1204
trial 3	0.1259
trial 4	0.1228
trial 5	0.1230
trial 6	0.1328
Average	0.1254
STD	0.0044

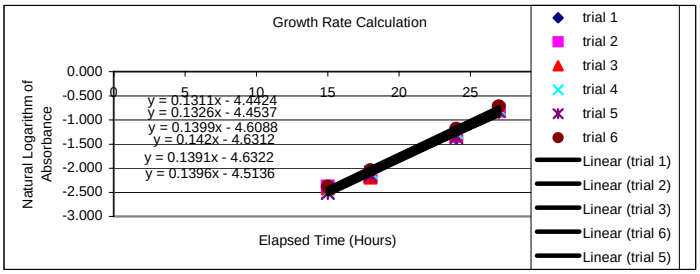


20 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.088	0.084	0.081	0.075	0.073	0.081	-2.429	-2.472	-2.518	-2.585	-2.615	-2.519
15	0.091	0.092	0.089	0.083	0.081	0.092	-2.402	-2.383	-2.422	-2.494	-2.512	-2.383
18	0.116	0.116	0.111	0.126	0.116	0.130	-2.158	-2.158	-2.196	-2.075	-2.151	-2.042
24	0.255	0.265	0.275	0.285	0.255	0.306	-1.368	-1.329	-1.292	-1.257	-1.365	-1.183
27	0.436	0.446	0.460	0.462	0.441	0.488	-0.831	-0.808	-0.776	-0.773	-0.819	-0.718
30	0.479	0.499	0.511	0.561	0.536	0.534	-0.736	-0.696	-0.672	-0.578	-0.623	-0.627
33	0.535	0.524	0.554	0.624	0.597	0.590	-0.626	-0.647	-0.591	-0.472	-0.515	-0.528
36	0.589	0.549	0.590	0.690	0.654	0.635	-0.529	-0.600	-0.528	-0.371	-0.424	-0.454
39	0.624	0.598	0.613	0.775	0.727	0.788	-0.472	-0.514	-0.490	-0.255	-0.319	-0.238
42	0.655	0.633	0.646	0.823	0.795	0.835	-0.423	-0.458	-0.438	-0.194	-0.230	-0.181
45	0.702	0.655	0.663	0.855	0.824	0.855	-0.353	-0.424	-0.412	-0.157	-0.194	-0.156
48	0.713	0.678	0.680	0.880	0.854	0.879	-0.339	-0.389	-0.386	-0.128	-0.157	-0.129
51	0.725	0.690	0.701	0.887	0.870	0.870	-0.322	-0.371	-0.356	-0.119	-0.140	-0.139
54	0.725	0.699	0.712	0.865	0.855	0.855	-0.321	-0.359	-0.339	-0.146	-0.157	-0.157
60	0.719	0.699	0.712	0.880	0.860	0.864	-0.330	-0.358	-0.340	-0.128	-0.151	-0.146

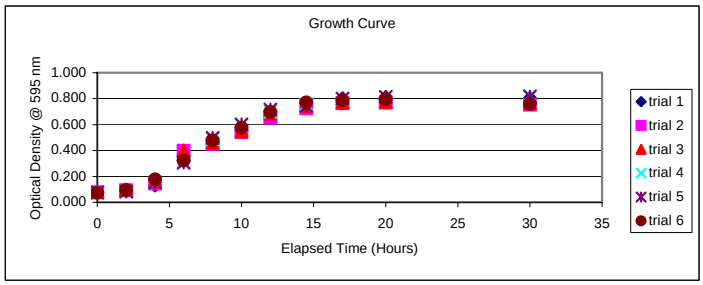


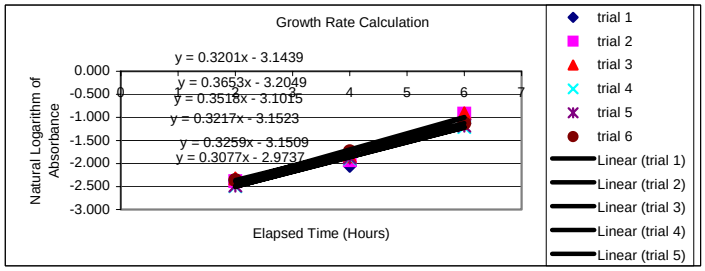
	Growth Rate
trial 1	0.1311
trial 2	0.1326
trial 3	0.1399
trial 4	0.1420
trial 5	0.1391
trial 6	0.1396
Average	0.1374
STD	0.0044



30 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.077	0.077	0.078	0.075	0.075	0.073	-2.570	-2.563	-2.550	-2.597	-2.588	-2.613
2	0.090	0.092	0.098	0.083	0.083	0.094	-2.411	-2.383	-2.322	-2.495	-2.485	-2.362
4	0.129	0.146	0.158	0.151	0.153	0.177	-2.049	-1.927	-1.846	-1.894	-1.875	-1.734
6	0.323	0.398	0.401	0.299	0.307	0.323	-1.131	-0.922	-0.915	-1.208	-1.182	-1.131
8	0.465	0.450	0.461	0.488	0.498	0.479	-0.767	-0.798	-0.774	-0.718	-0.698	-0.737
10	0.550	0.541	0.548	0.598	0.603	0.577	-0.598	-0.615	-0.601	-0.514	-0.507	-0.551
12	0.700	0.653	0.685	0.700	0.716	0.698	-0.356	-0.427	-0.379	-0.357	-0.335	-0.360
14.5	0.766	0.726	0.745	0.758	0.745	0.771	-0.267	-0.320	-0.294	-0.277	-0.295	-0.260
17	0.802	0.765	0.771	0.799	0.801	0.786	-0.220	-0.269	-0.261	-0.225	-0.222	-0.240
20	0.812	0.771	0.773	0.813	0.813	0.794	-0.208	-0.260	-0.257	-0.207	-0.207	-0.230
30	0.805	0.754	0.764	0.822	0.815	0.760	-0.217	-0.282	-0.269	-0.196	-0.204	-0.275

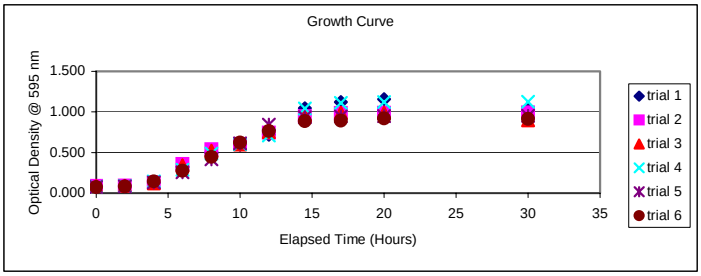




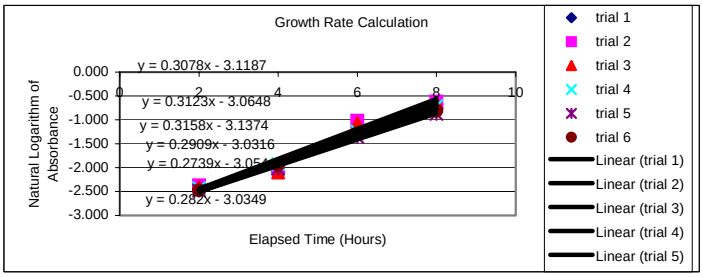
	Growth Rate
trial 1	0.3201
trial 2	0.3653
trial 3	0.3518
trial 4	0.3217
trial 5	0.3259
trial 6	0.3077
Average	0.3321
STD	0.0218

45 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.084	0.090	0.087	0.080	0.076	0.078	-2.473	-2.411	-2.442	-2.532	-2.582	-2.555
2	0.089	0.093	0.090	0.088	0.084	0.085	-2.422	-2.371	-2.412	-2.428	-2.481	-2.470
4	0.126	0.135	0.123	0.147	0.132	0.144	-2.075	-2.006	-2.100	-1.921	-2.022	-1.939
6	0.320	0.361	0.342	0.288	0.257	0.278	-1.140	-1.018	-1.073	-1.245	-1.360	-1.282
8	0.506	0.539	0.522	0.490	0.417	0.445	-0.682	-0.618	-0.650	-0.714	-0.875	-0.809
10	0.594	0.607	0.598	0.603	0.612	0.624	-0.520	-0.499	-0.514	-0.507	-0.491	-0.472
12	0.721	0.744	0.753	0.711	0.846	0.766	-0.327	-0.295	-0.284	-0.341	-0.168	-0.267
14.5	1.045	0.948	0.964	1.042	0.935	0.887	0.044	-0.053	-0.037	0.041	-0.068	-0.120
17	1.123	0.987	0.992	1.111	0.945	0.895	0.116	-0.013	-0.008	0.105	-0.056	-0.111
20	1.158	0.990	0.991	1.123	0.950	0.921	0.147	-0.010	-0.009	0.116	-0.051	-0.082
30	1.032	0.994	0.898	1.125	0.951	0.915	0.032	-0.006	-0.108	0.118	-0.050	-0.089

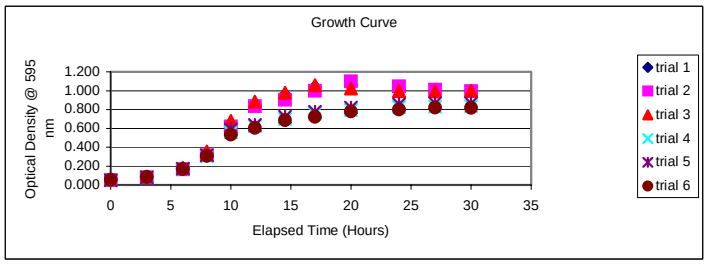


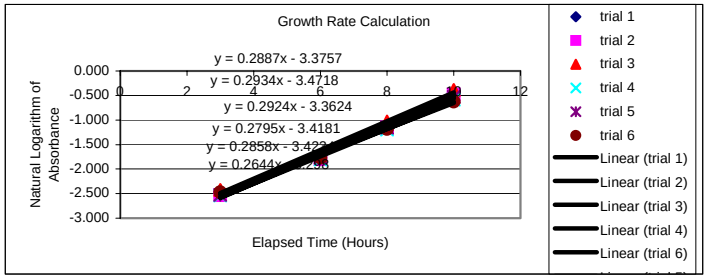
	Growth Rate
trial 1	0.3078
trial 2	0.3123
trial 3	0.3158
trial 4	0.2909
trial 5	0.2739
trial 6	0.2820
Average	0.2971
STD	0.0173



60 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.055	0.051	0.060	0.051	0.052	0.056	-2.893	-2.970	-2.817	-2.974	-2.966	-2.875
3	0.086	0.079	0.089	0.079	0.080	0.085	-2.458	-2.533	-2.422	-2.540	-2.528	-2.470
6	0.179	0.166	0.180	0.165	0.171	0.170	-1.721	-1.797	-1.714	-1.805	-1.766	-1.773
8	0.336	0.315	0.356	0.302	0.315	0.307	-1.091	-1.156	-1.032	-1.196	-1.156	-1.182
10	0.646	0.620	0.679	0.556	0.590	0.534	-0.437	-0.478	-0.387	-0.587	-0.528	-0.628
12	0.854	0.835	0.882	0.623	0.636	0.605	-0.158	-0.181	-0.125	-0.473	-0.453	-0.502
14.5	0.955	0.905	0.976	0.698	0.722	0.687	-0.046	-0.100	-0.024	-0.360	-0.326	-0.375
17	1.037	0.999	1.058	0.754	0.777	0.722	0.036	-0.001	0.056	-0.282	-0.253	-0.325
20	1.057	1.099	1.023	0.795	0.821	0.780	0.055	0.094	0.023	-0.229	-0.197	-0.249
24	1.002	1.046	0.995	0.827	0.857	0.801	0.002	0.045	-0.005	-0.191	-0.155	-0.223
27	0.999	1.012	0.991	0.831	0.864	0.822	-0.001	0.012	-0.009	-0.185	-0.146	-0.196
30	0.992	0.995	0.999	0.835	0.865	0.817	-0.008	-0.005	-0.001	-0.181	-0.145	-0.202

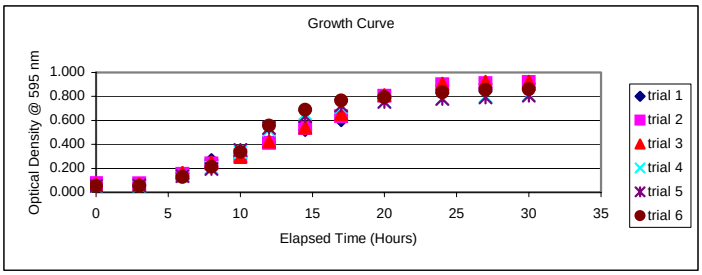




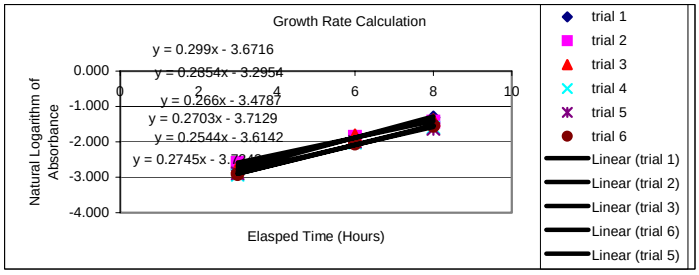
	Growth Rate
trial 1	0.2877
trial 2	0.2934
trial 3	0.2924
trial 4	0.2795
trial 5	0.2858
trial 6	0.2664
Average	0.2842
STD	0.0101

80 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.059	0.078	0.065	0.051	0.055	0.054	-2.825	-2.548	-2.727	-2.972	-2.908	-2.926
3	0.061	0.074	0.067	0.053	0.056	0.054	-2.794	-2.598	-2.708	-2.930	-2.890	-2.911
6	0.160	0.156	0.163	0.132	0.137	0.129	-1.831	-1.860	-1.816	-2.022	-1.991	-2.050
8	0.270	0.240	0.249	0.203	0.195	0.214	-1.308	-1.426	-1.391	-1.593	-1.637	-1.544
10	0.296	0.308	0.295	0.335	0.355	0.338	-1.216	-1.179	-1.219	-1.095	-1.037	-1.085
12	0.420	0.412	0.423	0.521	0.536	0.557	-0.868	-0.888	-0.860	-0.651	-0.623	-0.586
14.5	0.521	0.535	0.541	0.655	0.635	0.688	-0.653	-0.625	-0.614	-0.424	-0.455	-0.374
17	0.602	0.635	0.654	0.712	0.724	0.765	-0.507	-0.454	-0.424	-0.339	-0.324	-0.268
20	0.785	0.805	0.811	0.756	0.755	0.795	-0.243	-0.218	-0.209	-0.279	-0.282	-0.230
24	0.890	0.901	0.907	0.780	0.780	0.835	-0.117	-0.104	-0.098	-0.249	-0.248	-0.181
27	0.902	0.911	0.923	0.802	0.791	0.856	-0.103	-0.093	-0.080	-0.221	-0.234	-0.156
30	0.907	0.920	0.922	0.813	0.807	0.860	-0.098	-0.083	-0.082	-0.207	-0.215	-0.151

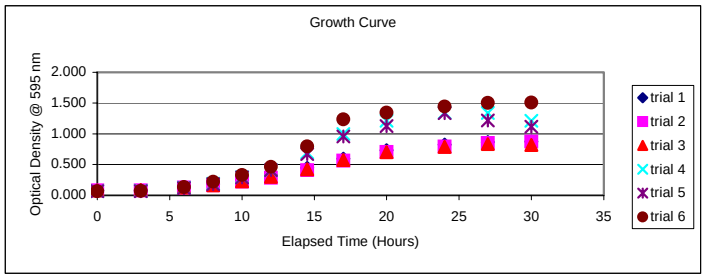


	Growth Rate
trial 1	0.2990
trial 2	0.2354
trial 3	0.2660
trial 4	0.2703
trial 5	0.2544
trial 6	0.2745
Average	0.2666
STD	0.0212

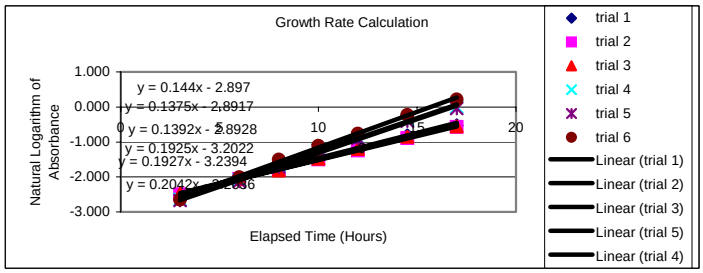


90 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.080	0.081	0.081	0.070	0.069	0.071	-2.521	-2.512	-2.515	-2.654	-2.679	-2.652
3	0.081	0.082	0.085	0.071	0.069	0.071	-2.512	-2.497	-2.471	-2.641	-2.674	-2.644
6	0.126	0.128	0.127	0.125	0.119	0.135	-2.071	-2.056	-2.060	-2.083	-2.129	-2.006
8	0.184	0.166	0.163	0.190	0.180	0.224	-1.694	-1.795	-1.817	-1.662	-1.713	-1.498
10	0.245	0.224	0.228	0.298	0.290	0.331	-1.408	-1.498	-1.479	-1.211	-1.236	-1.104
12	0.329	0.289	0.302	0.412	0.410	0.466	-1.112	-1.241	-1.197	-0.886	-0.892	-0.764
14.5	0.445	0.410	0.426	0.699	0.669	0.798	-0.809	-0.892	-0.854	-0.359	-0.402	-0.225
17	0.595	0.565	0.575	0.998	0.956	1.235	-0.520	-0.571	-0.554	-0.002	-0.045	0.211
20	0.735	0.706	0.701	1.213	1.124	1.345	-0.309	-0.348	-0.355	0.193	0.117	0.296
24	0.825	0.795	0.789	1.345	1.333	1.446	-0.192	-0.230	-0.236	0.296	0.287	0.369
27	0.865	0.854	0.835	1.341	1.215	1.505	-0.145	-0.158	-0.181	0.293	0.195	0.409
30	0.875	0.874	0.821	1.213	1.116	1.510	-0.134	-0.134	-0.197	0.193	0.110	0.412

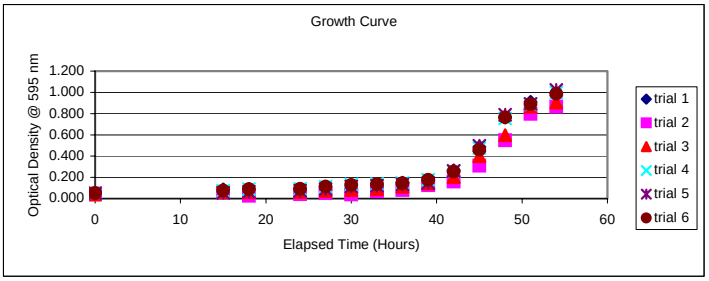


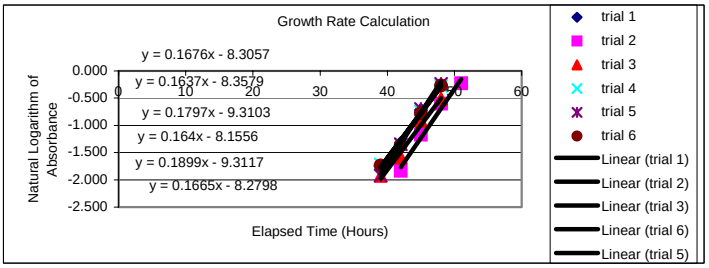
	Growth Rate
trial 1	0.144
trial 2	0.1375
trial 3	0.1392
trial 4	0.1925
trial 5	0.1927
trial 6	0.2042
Average	0.1684
STD	0.0312



100 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.047	0.046	0.040	0.051	0.050	0.053	-3.068	-3.073	-3.226	-2.972	-3.000	-2.930
15	0.087	0.058	0.060	0.075	0.055	0.077	-2.440	-2.846	-2.817	-2.596	-2.895	-2.563
18	0.088	0.027	0.061	0.088	0.063	0.089	-2.427	-3.627	-2.794	-2.428	-2.758	-2.416
24	0.073	0.043	0.064	0.091	0.075	0.091	-2.620	-3.156	-2.750	-2.394	-2.596	-2.397
27	0.085	0.053	0.075	0.112	0.090	0.113	-2.462	-2.930	-2.597	-2.187	-2.411	-2.177
30	0.082	0.040	0.082	0.135	0.113	0.128	-2.505	-3.229	-2.499	-2.000	-2.179	-2.053
33	0.102	0.067	0.092	0.139	0.121	0.136	-2.287	-2.697	-2.385	-1.974	-2.112	-1.992
36	0.150	0.081	0.112	0.147	0.135	0.146	-1.897	-2.516	-2.187	-1.921	-2.000	-1.928
39	0.175	0.126	0.146	0.180	0.147	0.177	-1.744	-2.070	-1.927	-1.716	-1.921	-1.734
42	0.266	0.160	0.204	0.259	0.260	0.255	-1.324	-1.831	-1.592	-1.352	-1.346	-1.368
45	0.488	0.310	0.400	0.476	0.498	0.460	-0.718	-1.170	-0.917	-0.742	-0.698	-0.776
48	0.764	0.550	0.597	0.756	0.788	0.766	-0.269	-0.599	-0.515	-0.279	-0.238	-0.267
51	0.910	0.798	0.865	0.887	0.891	0.890	-0.094	-0.225	-0.146	-0.120	-0.116	-0.116
54	0.994	0.865	0.905	0.999	1.023	0.988	-0.006	-0.146	-0.099	-0.001	0.023	-0.013
60	1.021	0.890	0.912	0.997	1.050	0.984	0.021	-0.117	-0.092	-0.004	0.049	-0.017

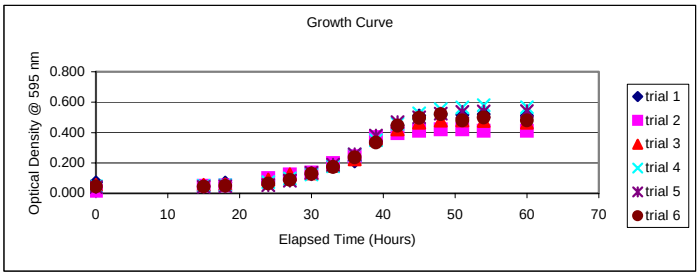




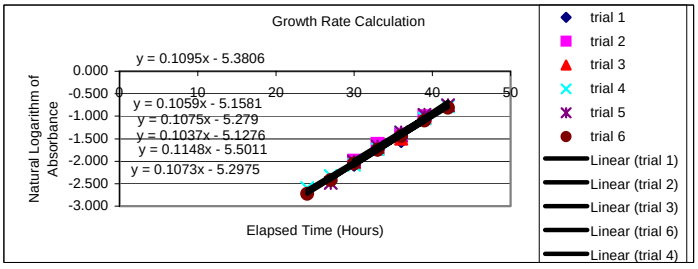
	Growth Rate
trial 1	0.1676
trial 2	0.1797
trial 3	0.1637
trial 4	0.164
trial 5	0.1899
trial 6	0.1665
Average	0.1719
STD	0.0106

115 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.073	0.017	0.055	0.046	0.041	0.045	-2.619	-4.069	-2.906	-3.088	-3.189	-3.112
15	0.046	0.049	0.055	0.050	0.042	0.045	-3.086	-3.008	-2.904	-3.002	-3.163	-3.103
18	0.071	0.052	0.060	0.054	0.046	0.050	-2.649	-2.953	-2.818	-2.926	-3.088	-3.006
24	0.082	0.099	0.091	0.075	0.054	0.066	-2.506	-2.314	-2.402	-2.596	-2.928	-2.726
27	0.121	0.123	0.125	0.098	0.084	0.089	-2.114	-2.094	-2.076	-2.327	-2.483	-2.422
30	0.125	0.137	0.131	0.125	0.135	0.129	-2.077	-1.989	-2.036	-2.083	-2.006	-2.049
33	0.175	0.199	0.180	0.177	0.188	0.174	-1.742	-1.614	-1.714	-1.732	-1.672	-1.748
36	0.212	0.244	0.224	0.255	0.257	0.237	-1.554	-1.411	-1.496	-1.368	-1.359	-1.440
39	0.352	0.369	0.356	0.350	0.380	0.335	-1.045	-0.998	-1.034	-1.050	-0.968	-1.095
42	0.447	0.396	0.421	0.458	0.469	0.446	-0.805	-0.927	-0.865	-0.781	-0.758	-0.809
45	0.512	0.412	0.466	0.526	0.501	0.498	-0.669	-0.886	-0.764	-0.642	-0.692	-0.698
48	0.521	0.422	0.479	0.555	0.525	0.521	-0.651	-0.864	-0.736	-0.590	-0.645	-0.651
51	0.517	0.421	0.481	0.565	0.535	0.480	-0.660	-0.866	-0.733	-0.571	-0.626	-0.734
54	0.521	0.411	0.478	0.580	0.540	0.499	-0.651	-0.888	-0.739	-0.545	-0.616	-0.695
60	0.511	0.411	0.466	0.568	0.542	0.481	-0.671	-0.889	-0.764	-0.566	-0.613	-0.732

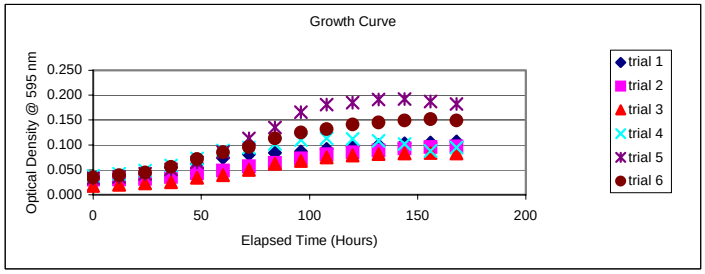


	Growth Rate
trial 1	0.1095
trial 2	0.1059
trial 3	0.1075
trial 4	0.1037
trial 5	0.1148
trial 6	0.1073
Average	0.1081
STD	0.0038

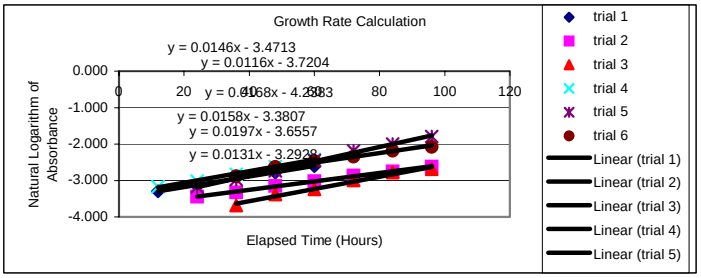


145 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.034	0.030	0.018	0.038	0.033	0.035	-3.381	-3.507	-4.017	-3.270	-3.411	-3.352
12	0.037	0.031	0.020	0.042	0.036	0.039	-3.297	-3.474	-3.912	-3.170	-3.324	-3.244
24	0.043	0.032	0.023	0.049	0.042	0.045	-3.147	-3.442	-3.772	-3.016	-3.170	-3.101
36	0.055	0.036	0.025	0.059	0.051	0.056	-2.900	-3.324	-3.689	-2.830	-2.976	-2.882
48	0.062	0.043	0.034	0.073	0.065	0.072	-2.781	-3.147	-3.381	-2.617	-2.733	-2.631
60	0.074	0.049	0.039	0.089	0.086	0.085	-2.604	-3.016	-3.244	-2.419	-2.453	-2.465
72	0.081	0.057	0.050	0.095	0.113	0.096	-2.513	-2.865	-2.996	-2.354	-2.180	-2.343
84	0.084	0.064	0.062	0.102	0.135	0.113	-2.477	-2.749	-2.781	-2.283	-2.002	-2.180
96	0.087	0.073	0.068	0.110	0.166	0.125	-2.442	-2.617	-2.688	-2.207	-1.796	-2.079
108	0.092	0.081	0.075	0.113	0.181	0.132	-2.386	-2.513	-2.590	-2.180	-1.709	-2.025
120	0.097	0.085	0.079	0.112	0.185	0.141	-2.333	-2.465	-2.538	-2.189	-1.687	-1.959
132	0.099	0.089	0.082	0.108	0.191	0.145	-2.313	-2.419	-2.501	-2.226	-1.655	-1.931
144	0.103	0.093	0.083	0.102	0.192	0.149	-2.273	-2.375	-2.489	-2.283	-1.650	-1.904
156	0.105	0.096	0.084	0.088	0.187	0.152	-2.254	-2.343	-2.477	-2.430	-1.677	-1.884
168	0.107	0.097	0.083	0.095	0.182	0.149	-2.235	-2.333	-2.489	-2.354	-1.704	-1.904

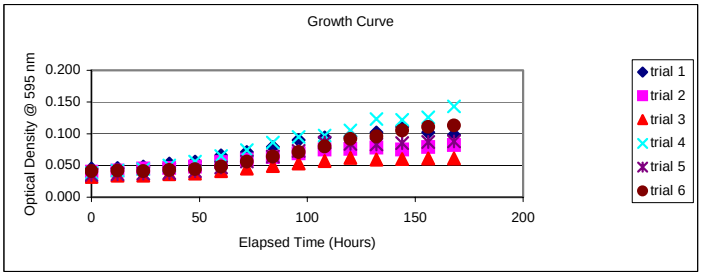


	Growth Rate
trial 1	0.0146
trial 2	0.0116
trial 3	0.0168
trial 4	0.0158
trial 5	0.0197
trial 6	0.0131
Average	0.0153
STD	0.0029

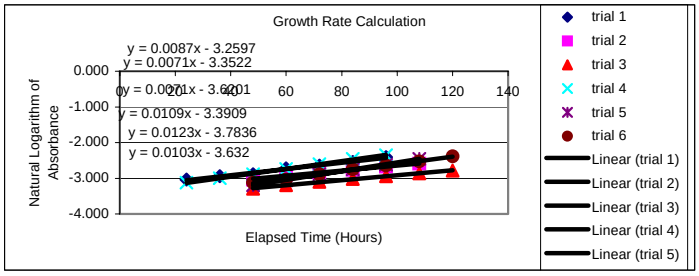


175 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.045	0.040	0.032	0.038	0.035	0.041	-3.101	-3.219	-3.442	-3.270	-3.352	-3.194
12	0.046	0.042	0.034	0.042	0.036	0.042	-3.079	-3.170	-3.381	-3.170	-3.324	-3.170
24	0.048	0.045	0.034	0.044	0.037	0.041	-3.037	-3.101	-3.381	-3.124	-3.297	-3.194
36	0.053	0.046	0.036	0.050	0.038	0.043	-2.937	-3.079	-3.324	-2.996	-3.270	-3.147
48	0.056	0.048	0.037	0.056	0.041	0.044	-2.882	-3.037	-3.297	-2.882	-3.194	-3.124
60	0.066	0.055	0.041	0.065	0.047	0.048	-2.718	-2.900	-3.194	-2.733	-3.058	-3.037
72	0.071	0.059	0.045	0.074	0.056	0.056	-2.645	-2.830	-3.101	-2.604	-2.882	-2.882
84	0.079	0.063	0.049	0.086	0.064	0.064	-2.538	-2.765	-3.016	-2.453	-2.749	-2.749
96	0.090	0.069	0.053	0.095	0.073	0.071	-2.408	-2.674	-2.937	-2.354	-2.617	-2.645
108	0.094	0.075	0.057	0.097	0.086	0.080	-2.364	-2.590	-2.865	-2.333	-2.453	-2.526
120	0.096	0.076	0.062	0.105	0.084	0.092	-2.343	-2.577	-2.781	-2.254	-2.477	-2.386
132	0.102	0.078	0.059	0.123	0.083	0.096	-2.283	-2.551	-2.830	-2.096	-2.489	-2.343
144	0.111	0.075	0.060	0.122	0.085	0.105	-2.198	-2.590	-2.813	-2.104	-2.465	-2.254
156	0.102	0.079	0.061	0.126	0.087	0.111	-2.283	-2.538	-2.797	-2.071	-2.442	-2.198
168	0.098	0.082	0.061	0.143	0.088	0.113	-2.323	-2.501	-2.797	-1.945	-2.430	-2.180

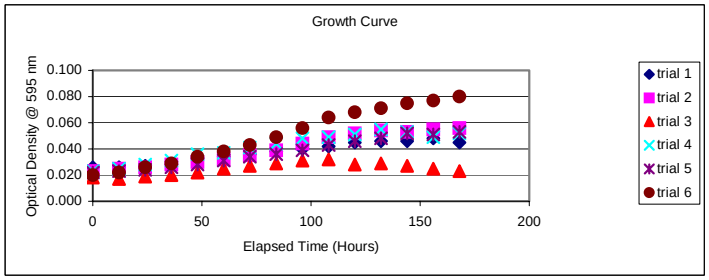


	Growth Rate
trial 1	0.0087
trial 2	0.0071
trial 3	0.0071
trial 4	0.0109
trial 5	0.0123
trial 6	0.0103
Average	0.0094
STD	0.0021

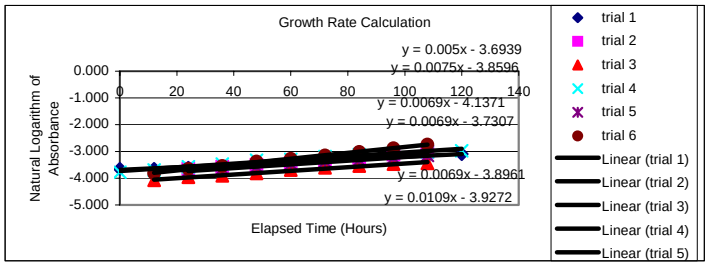


205 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.026	0.023	0.018	0.023	0.022	0.020	-3.650	-3.772	-4.017	-3.772	-3.817	-3.912
12	0.026	0.025	0.017	0.025	0.023	0.022	-3.650	-3.689	-4.075	-3.689	-3.772	-3.817
24	0.027	0.026	0.019	0.028	0.024	0.026	-3.612	-3.650	-3.963	-3.576	-3.730	-3.650
36	0.028	0.028	0.020	0.031	0.026	0.029	-3.576	-3.576	-3.912	-3.474	-3.650	-3.540
48	0.033	0.030	0.022	0.036	0.028	0.034	-3.411	-3.507	-3.817	-3.324	-3.576	-3.381
60	0.034	0.032	0.025	0.037	0.031	0.038	-3.381	-3.442	-3.689	-3.297	-3.474	-3.270
72	0.036	0.035	0.027	0.041	0.034	0.043	-3.324	-3.352	-3.612	-3.194	-3.381	-3.147
84	0.038	0.039	0.029	0.044	0.036	0.049	-3.270	-3.244	-3.540	-3.124	-3.324	-3.016
96	0.040	0.044	0.031	0.048	0.039	0.056	-3.219	-3.124	-3.474	-3.037	-3.244	-2.882
108	0.042	0.049	0.032	0.049	0.043	0.064	-3.170	-3.016	-3.442	-3.016	-3.147	-2.749
120	0.045	0.052	0.028	0.051	0.046	0.068	-3.101	-2.957	-3.576	-2.976	-3.079	-2.688
132	0.046	0.054	0.029	0.055	0.048	0.071	-3.079	-2.919	-3.540	-2.900	-3.037	-2.645
144	0.046	0.053	0.027	0.053	0.052	0.075	-3.079	-2.937	-3.612	-2.937	-2.957	-2.590
156	0.048	0.055	0.025	0.049	0.051	0.077	-3.037	-2.900	-3.689	-3.016	-2.976	-2.564
168	0.045	0.056	0.023	0.052	0.053	0.080	-3.101	-2.882	-3.772	-2.957	-2.937	-2.526

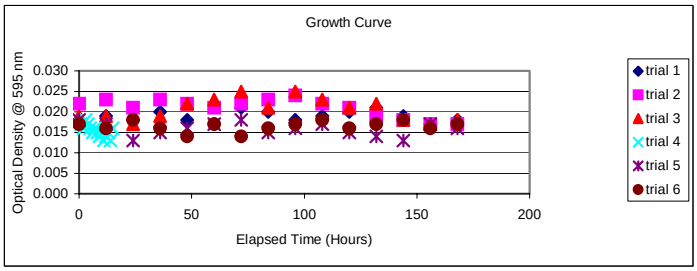


	Growth Rate
trial 1	0.0050
trial 2	0.0075
trial 3	0.0069
trial 4	0.0069
trial 5	0.0069
trial 6	0.0109
Average	0.0074
STD	0.0019



235 grams/liter

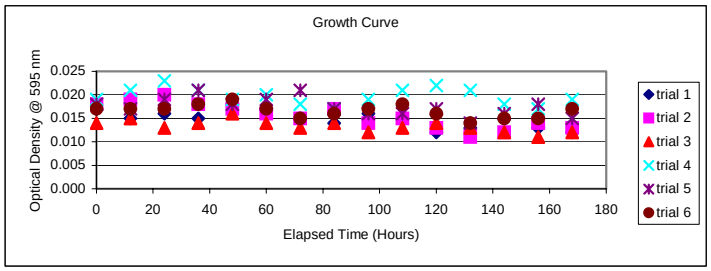
							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.018	0.022	0.019	0.017	0.018	0.017	-4.017	-3.817	-3.963	-4.075	-4.017	-4.075
12	0.019	0.023	0.019	0.016	0.017	0.016	-3.963	-3.772	-3.963	-4.135	-4.075	-4.135
24	0.018	0.021	0.017	0.018	0.013	0.018	-4.017	-3.863	-4.075	-4.017	-4.343	-4.017
36	0.020	0.023	0.019	0.017	0.015	0.016	-3.912	-3.772	-3.963	-4.075	-4.200	-4.135
48	0.018	0.022	0.022	0.016	0.016	0.014	-4.017	-3.817	-3.817	-4.135	-4.135	-4.269
60	0.017	0.021	0.023	0.015	0.017	0.017	-4.075	-3.863	-3.772	-4.200	-4.075	-4.075
72	0.021	0.022	0.025	0.016	0.018	0.014	-3.863	-3.817	-3.689	-4.135	-4.017	-4.269
84	0.020	0.023	0.021	0.015	0.015	0.016	-3.912	-3.772	-3.863	-4.200	-4.200	-4.135
96	0.018	0.024	0.025	0.014	0.016	0.017	-4.017	-3.730	-3.689	-4.269	-4.135	-4.075
108	0.019	0.022	0.023	0.015	0.017	0.018	-3.963	-3.817	-3.772	-4.200	-4.075	-4.017
120	0.020	0.021	0.021	0.013	0.015	0.016	-3.912	-3.863	-3.863	-4.343	-4.200	-4.135
132	0.021	0.019	0.022	0.014	0.014	0.017	-3.863	-3.963	-3.817	-4.269	-4.269	-4.075
144	0.019	0.018	0.018	0.015	0.013	0.018	-3.963	-4.017	-4.017	-4.200	-4.343	-4.017
156	0.017	0.017	0.017	0.013	0.017	0.016	-4.075	-4.075	-4.075	-4.343	-4.075	-4.135
168	0.018	0.017	0.018	0.016	0.016	0.017	-4.017	-4.075	-4.017	-4.135	-4.135	-4.075



	Growth Rate
trial 1	0
trial 2	0
trial 3	0
trial 4	0
trial 5	0
trial 6	0
Average	0
STD	0.0000

260 grams/liter

							Logarithms					
elapsed time	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6	trial 1	trial 2	trial 3	trial 4	trial 5	trial 6
0	0.017	0.018	0.014	0.019	0.018	0.017	-4.075	-4.017	-4.269	-3.963	-4.017	-4.075
12	0.015	0.019	0.015	0.021	0.017	0.017	-4.200	-3.963	-4.200	-3.863	-4.075	-4.075
24	0.016	0.020	0.013	0.023	0.019	0.017	-4.135	-3.912	-4.343	-3.772	-3.963	-4.075
36	0.015	0.018	0.014	0.021	0.021	0.018	-4.200	-4.017	-4.269	-3.863	-3.863	-4.017
48	0.018	0.017	0.016	0.019	0.018	0.019	-4.017	-4.075	-4.135	-3.963	-4.017	-3.963
60	0.017	0.016	0.014	0.020	0.019	0.017	-4.075	-4.135	-4.269	-3.912	-3.963	-4.075
72	0.015	0.015	0.013	0.018	0.021	0.015	-4.200	-4.200	-4.343	-4.017	-3.863	-4.200
84	0.014	0.017	0.014	0.017	0.017	0.016	-4.269	-4.075	-4.269	-4.075	-4.075	-4.135
96	0.016	0.014	0.012	0.019	0.016	0.017	-4.135	-4.269	-4.423	-3.963	-4.135	-4.075
108	0.015	0.015	0.013	0.021	0.016	0.018	-4.200	-4.200	-4.343	-3.863	-4.135	-4.017
120	0.012	0.013	0.014	0.022	0.017	0.016	-4.423	-4.343	-4.269	-3.817	-4.075	-4.135
132	0.013	0.011	0.013	0.021	0.014	0.014	-4.343	-4.510	-4.343	-3.863	-4.269	-4.269
144	0.015	0.012	0.012	0.018	0.016	0.015	-4.200	-4.423	-4.423	-4.017	-4.135	-4.200
156	0.013	0.014	0.011	0.017	0.018	0.015	-4.343	-4.269	-4.510	-4.075	-4.017	-4.200
168	0.012	0.013	0.012	0.019	0.015	0.017	-4.423	-4.343	-4.423	-3.963	-4.200	-4.075



	Growth Rate
trial 1	0
trial 2	0
trial 3	0
trial 4	0
trial 5	0
trial 6	0
Average	0
STD	0.0000

Appendix F

Appendix F details data collected from experiments designed to test the ability of *H. campisalis* to uptake the compatible solute ectoine from the environment. Growth kinetics were determined in the presence of both glucose and ectoine. Additionally, the ability of *H. campisalis* to utilize ectoine as a sole carbon source was tested. 1 g/L of ectoine was used in each experiment, as well as 0.5 g/L of glucose. Glucose was reduced in order to more clearly discern the effects of the compatible solutes.

Effect of media spiking using ectoine on the growth kinetics of *H. campisalis*

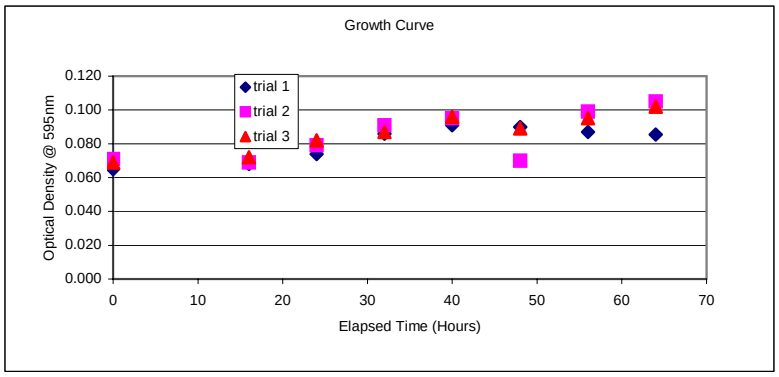
Total Ectoine Substrate Data

	20 g/L			60 g/L	
Substrate	Growth Rate	STD	Substrate	Growth Rate	STD
Ectoine	0.0086	0.0004	Ectoine	0.0118	0.0019
Glucose	0.1157	0.0105	Glucose	0.2208	0.0202
Both	0.1018	0.0047	Both	0.2084	0.0078

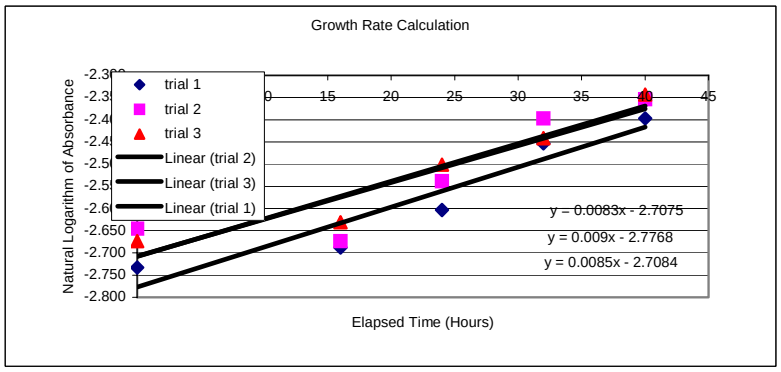
Ectoine

20 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.065	0.071	0.069	-2.733	-2.645	-2.674
16	0.068	0.069	0.072	-2.688	-2.674	-2.631
24	0.074	0.079	0.082	-2.604	-2.538	-2.501
32	0.086	0.091	0.087	-2.453	-2.397	-2.442
40	0.091	0.095	0.096	-2.397	-2.354	-2.343
48	0.090	0.070	0.089	-2.408	-2.659	-2.419
56	0.087	0.099	0.095	-2.442	-2.313	-2.354
64	0.085	0.105	0.102	-2.460	-2.254	-2.283



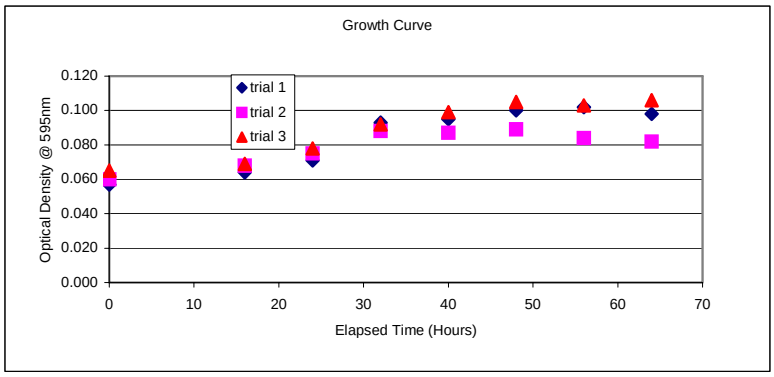
	Growth Rate
trial 1	0.0090
trial 2	0.0083
trial 3	0.0085
average	0.0086
std	0.0004



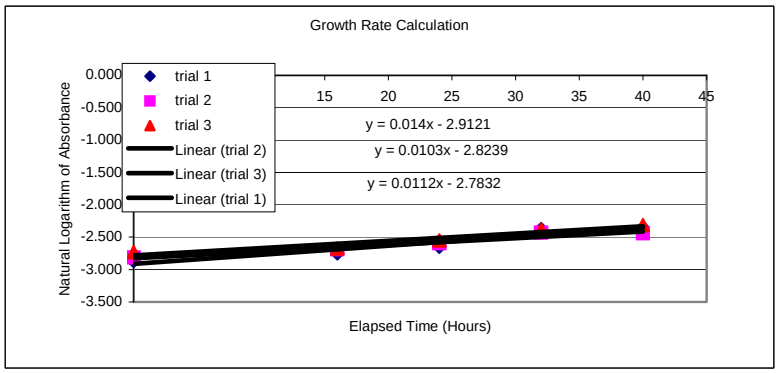
Ectoine

60 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.057	0.060	0.065	-2.865	-2.813	-2.733
16	0.064	0.068	0.069	-2.749	-2.688	-2.674
24	0.071	0.075	0.078	-2.645	-2.590	-2.551
32	0.093	0.088	0.092	-2.375	-2.430	-2.386
40	0.095	0.087	0.099	-2.354	-2.442	-2.313
48	0.100	0.089	0.105	-2.303	-2.419	-2.254
56	0.102	0.084	0.103	-2.283	-2.477	-2.273
64	0.098	0.082	0.106	-2.323	-2.501	-2.244



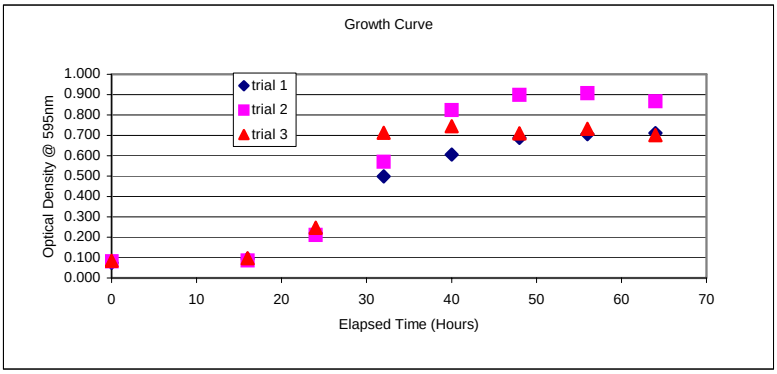
	Growth Rate
trial 1	0.0140
trial 2	0.0103
trial 3	0.0112
average	0.0118
std	0.0019



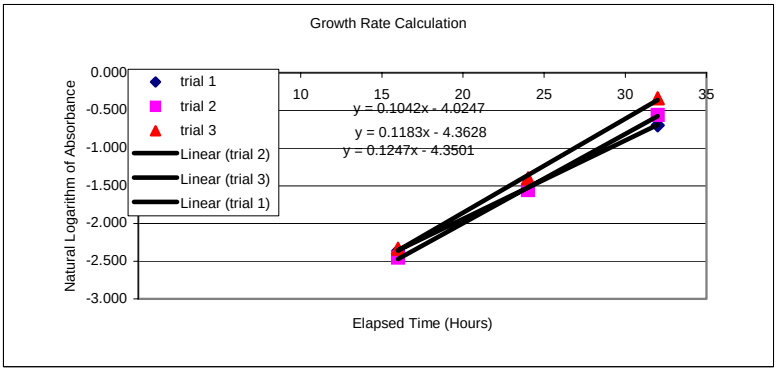
Glucose

20 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.074	0.082	0.085	-2.604	-2.501	-2.465
16	0.094	0.086	0.097	-2.364	-2.453	-2.333
24	0.221	0.211	0.246	-1.510	-1.556	-1.402
32	0.498	0.571	0.713	-0.697	-0.560	-0.338
40	0.605	0.824	0.745	-0.503	-0.194	-0.294
48	0.687	0.898	0.710	-0.375	-0.108	-0.342
56	0.705	0.907	0.733	-0.350	-0.098	-0.311
64	0.711	0.867	0.702	-0.341	-0.143	-0.354



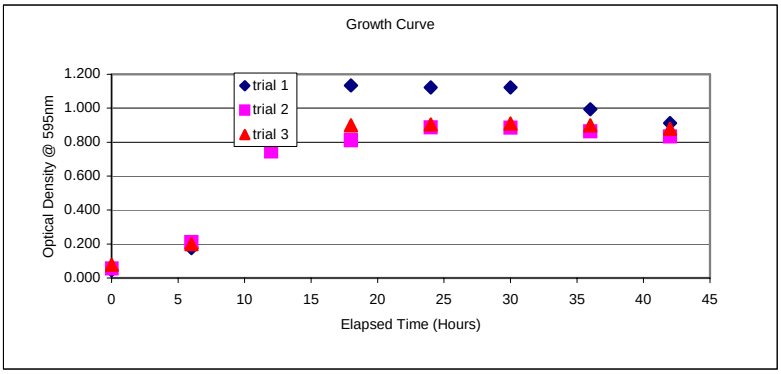
	Growth Rate
trial 1	0.1042
trial 2	0.1183
trial 3	0.1247
average	0.1157
std	0.0105



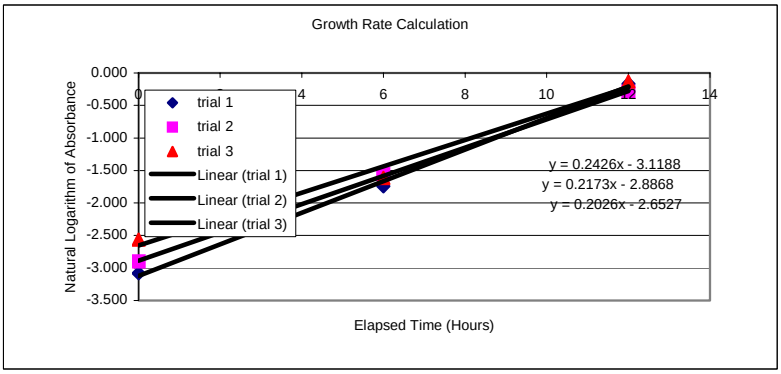
Glucose

60 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.046	0.055	0.077	-3.079	-2.900	-2.564
6	0.175	0.211	0.199	-1.743	-1.556	-1.614
12	0.845	0.746	0.876	-0.168	-0.293	-0.132
18	1.134	0.812	0.899	0.126	-0.208	-0.106
24	1.123	0.887	0.905	0.116	-0.120	-0.100
30	1.123	0.885	0.910	0.116	-0.122	-0.094
36	0.994	0.864	0.900	-0.006	-0.146	-0.105
42	0.913	0.833	0.879	-0.091	-0.183	-0.129



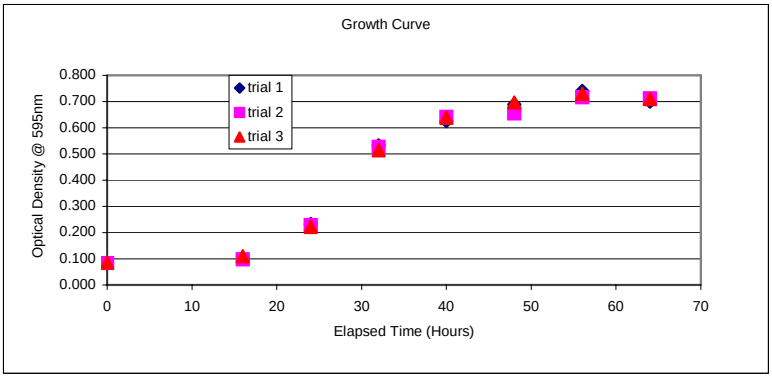
	Growth Rate
trial 1	0.2426
trial 2	0.2173
trial 3	0.2026
average	0.2208
std	0.0202



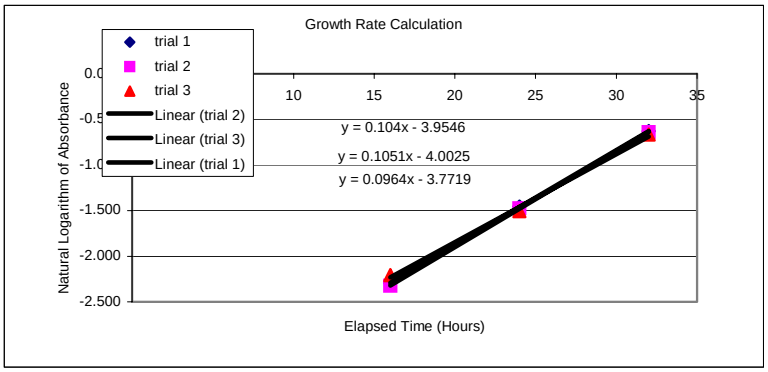
Both

20 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.083	0.084	0.086	-2.489	-2.477	-2.453
16	0.101	0.098	0.110	-2.293	-2.323	-2.207
24	0.233	0.229	0.222	-1.457	-1.474	-1.505
32	0.533	0.527	0.514	-0.629	-0.641	-0.666
40	0.625	0.642	0.639	-0.470	-0.443	-0.448
48	0.689	0.655	0.697	-0.373	-0.423	-0.361
56	0.741	0.717	0.730	-0.300	-0.333	-0.315
64	0.700	0.712	0.710	-0.357	-0.340	-0.342



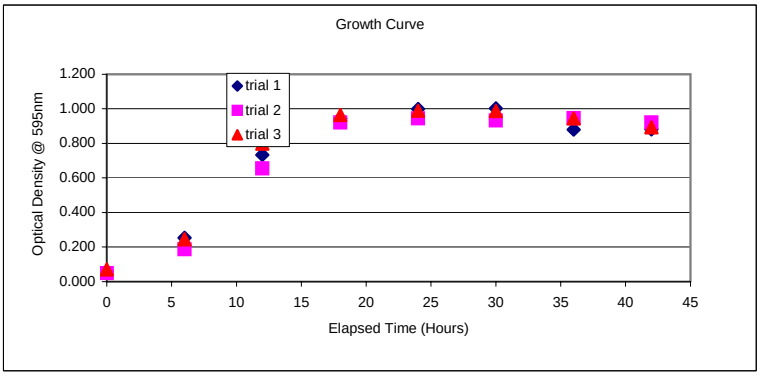
	Growth Rate
trial 1	0.1040
trial 2	0.1051
trial 3	0.0964
average	0.1018
std	0.0047



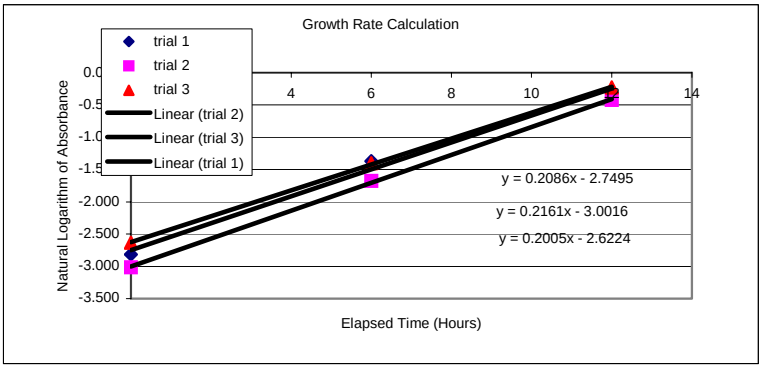
Both

60 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.060	0.049	0.072	-2.813	-3.016	-2.631
6	0.254	0.187	0.246	-1.370	-1.677	-1.402
12	0.733	0.655	0.798	-0.311	-0.423	-0.226
18	0.945	0.921	0.965	-0.057	-0.082	-0.036
24	0.999	0.945	0.990	-0.001	-0.057	-0.010
30	1.002	0.933	0.987	0.002	-0.069	-0.013
36	0.879	0.946	0.945	-0.129	-0.056	-0.057
42	0.882	0.921	0.893	-0.126	-0.082	-0.113



	Growth Rate
trial 1	0.2086
trial 2	0.2161
trial 3	0.2005
average	0.2084
std	0.0078



Appendix G

Appendix G details data collected from experiments designed to test the ability of *H. campisalis* to uptake the compatible solute glycine betaine from the environment. Growth kinetics were determined in the presence of both glucose and glycine betaine. Additionally, the ability of *H. campisalis* to utilize glycine betaine as a sole carbon source was tested. 1 g/L of glycine betaine was used in each experiment, as well as 0.5 g/L of glucose. Glucose was reduced in order to more clearly discern the effects of the compatible solutes. Although glucose only controls were used both in the data presented in appendix f and in the data presented here, they were combined in the plots shown in Chapter 2 (Figures 12 and 13).

Effect of media spiking using glycine betaine on the growth of *H. campisalis*

Total Glycine Betaine Substrate Data

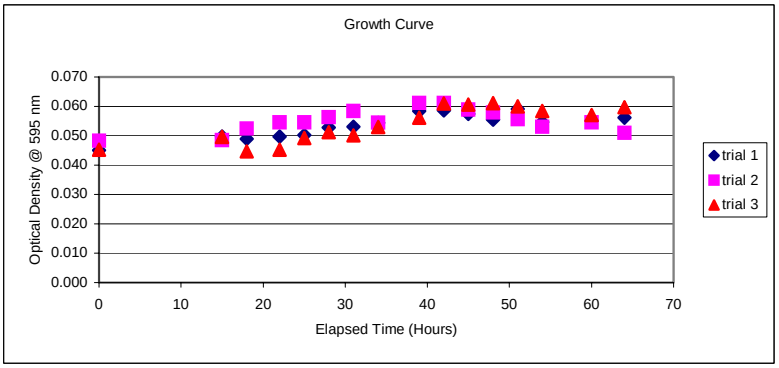
	20 g/L	
Substrate	Growth Rate	STD
Glycine Betaine	0.0088	0.0031
Glucose	0.1635	0.0176
Both	0.1669	0.0175

	60 g/L	
Substrate	Growth Rate	STD
Glycine Betaine	0.0334	0.0002
Glucose	0.2839	0.0199
Both	0.3054	0.0164

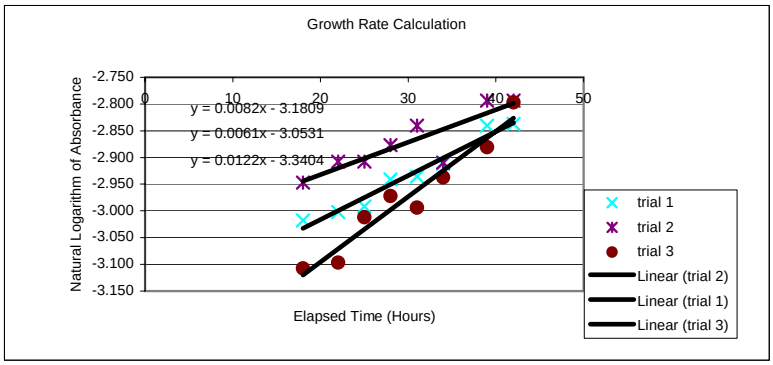
Glycine Betaine

20 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.045	0.048	0.045	-3.101	-3.030	-3.097
15	0.050	0.049	0.050	-3.002	-3.026	-3.004
18	0.049	0.053	0.045	-3.018	-2.947	-3.108
22	0.050	0.055	0.045	-3.002	-2.908	-3.097
25	0.050	0.055	0.049	-2.992	-2.908	-3.012
28	0.053	0.056	0.051	-2.941	-2.877	-2.972
31	0.053	0.058	0.050	-2.936	-2.840	-2.994
34	0.054	0.055	0.053	-2.911	-2.910	-2.937
39	0.058	0.061	0.056	-2.840	-2.794	-2.881
42	0.059	0.061	0.061	-2.837	-2.794	-2.797
45	0.057	0.059	0.061	-2.858	-2.832	-2.803
48	0.055	0.058	0.061	-2.893	-2.849	-2.795
51	0.059	0.056	0.060	-2.829	-2.890	-2.813
54	0.055	0.053	0.058	-2.906	-2.936	-2.840
60	0.055	0.055	0.057	-2.899	-2.908	-2.865
64	0.056	0.051	0.060	-2.881	-2.976	-2.818



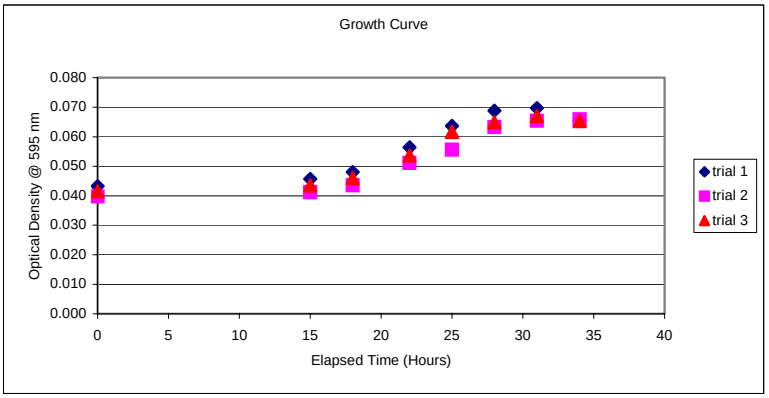
	Growth Rate
trial 1	0.0082
tiral 2	0.0061
trial 3	0.0122
average	0.0088
std	0.0031



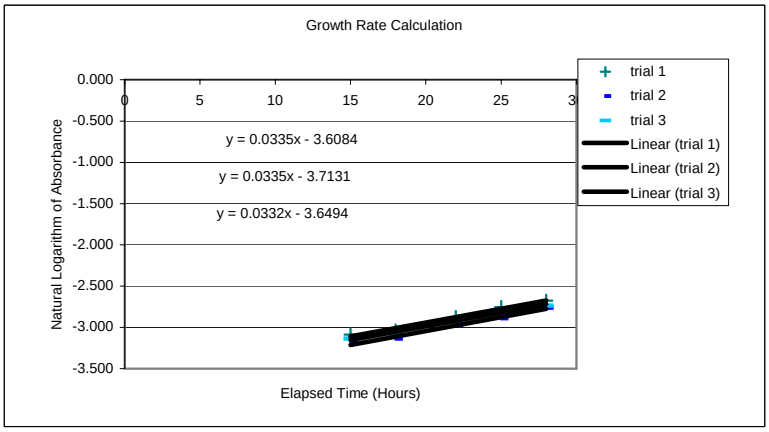
Glycine Betaine

60 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.043	0.040	0.042	-3.140	-3.224	-3.182
15	0.046	0.041	0.044	-3.086	-3.189	-3.135
18	0.048	0.044	0.046	-3.037	-3.135	-3.079
22	0.056	0.051	0.054	-2.875	-2.974	-2.924
25	0.064	0.056	0.062	-2.754	-2.890	-2.787
28	0.069	0.063	0.065	-2.677	-2.760	-2.735
31	0.070	0.065	0.067	-2.664	-2.727	-2.703
34	0.066	0.066	0.065	-2.726	-2.720	-2.729



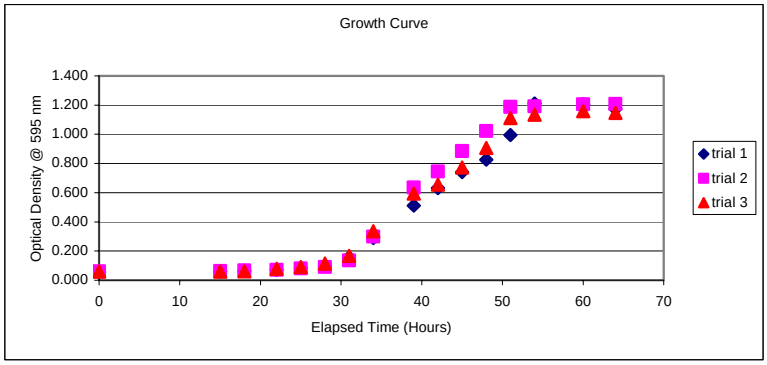
	Growth Rate
trial 1	0.0335
tiral 2	0.0335
trial 3	0.0332
average	0.0334
std	0.0002



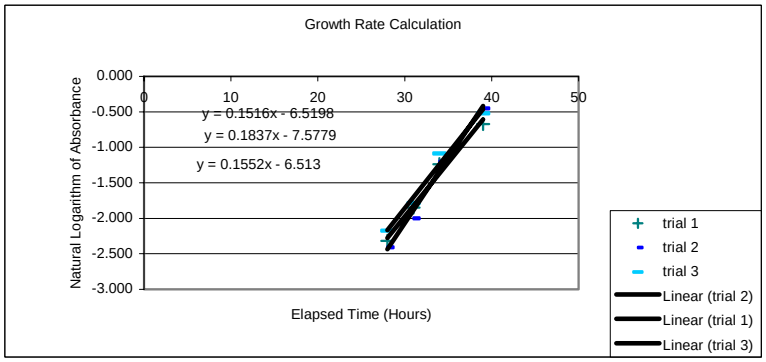
Glucose

20 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.055	0.061	0.059	-2.904	-2.794	-2.832
15	0.059	0.062	0.060	-2.832	-2.777	-2.817
18	0.064	0.067	0.062	-2.743	-2.709	-2.789
22	0.071	0.071	0.078	-2.642	-2.649	-2.555
25	0.084	0.080	0.090	-2.477	-2.523	-2.406
28	0.099	0.090	0.114	-2.316	-2.408	-2.176
31	0.158	0.135	0.168	-1.846	-2.003	-1.786
34	0.290	0.300	0.337	-1.239	-1.205	-1.089
39	0.511	0.636	0.594	-0.671	-0.453	-0.520
42	0.631	0.746	0.654	-0.460	-0.293	-0.424
45	0.741	0.885	0.773	-0.300	-0.123	-0.257
48	0.826	1.023	0.907	-0.192	0.023	-0.098
51	0.995	1.188	1.111	-0.005	0.172	0.105
54	1.211	1.192	1.134	0.191	0.176	0.126
60	1.205	1.206	1.159	0.186	0.187	0.147
64	1.173	1.208	1.145	0.160	0.189	0.136



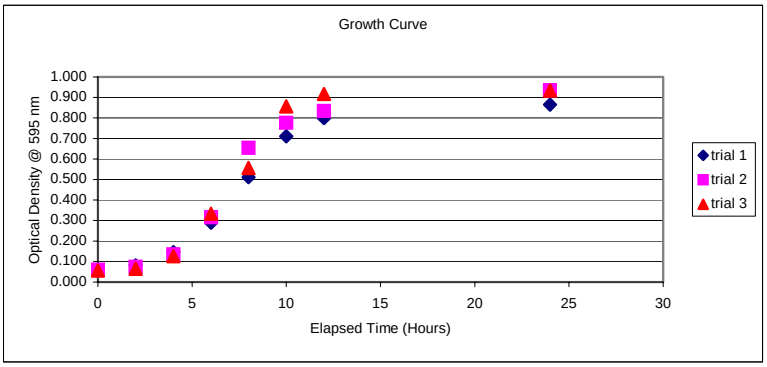
	Growth Rate
trial 1	0.1516
tiral 2	0.1837
trial 3	0.1552
average	0.1635
std	0.0176



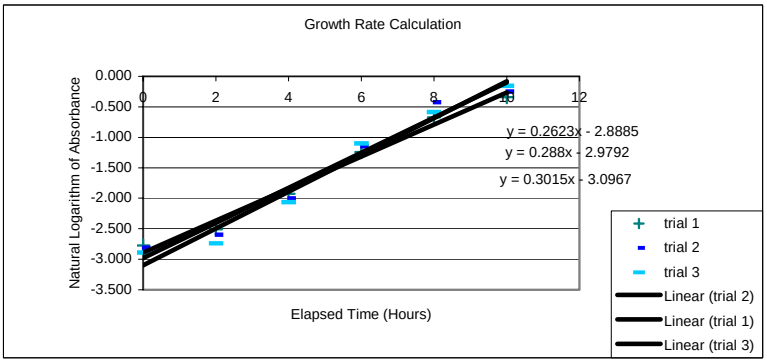
Glucose

60 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.062	0.060	0.056	-2.774	-2.810	-2.890
2	0.082	0.075	0.065	-2.507	-2.596	-2.741
4	0.146	0.135	0.127	-1.927	-2.004	-2.065
6	0.289	0.317	0.333	-1.242	-1.150	-1.099
8	0.512	0.655	0.556	-0.670	-0.423	-0.586
10	0.711	0.777	0.856	-0.341	-0.252	-0.155
12	0.799	0.835	0.917	-0.225	-0.181	-0.087
24	0.865	0.935	0.935	-0.145	-0.067	-0.068

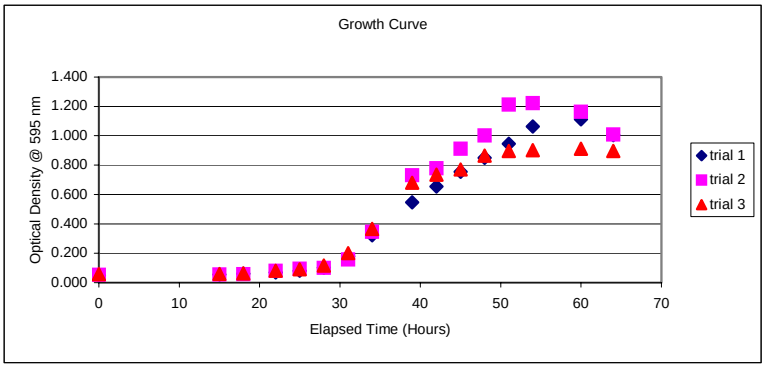


	Growth Rate
trial 1	0.2623
tiral 2	0.2880
trial 3	0.3015
average	0.2839
std	0.0199

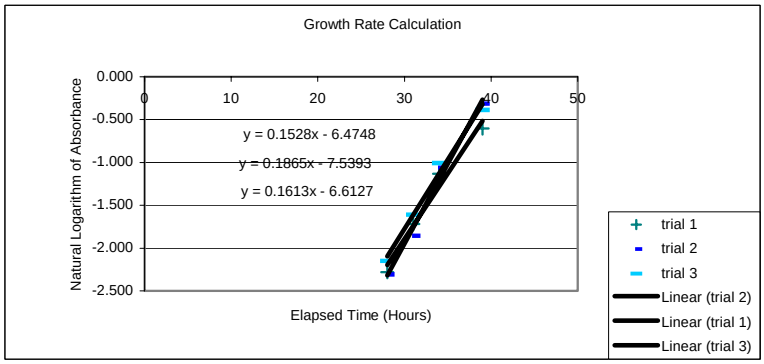


Both 20 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.050	0.052	0.056	-3.002	-2.953	-2.875
15	0.056	0.055	0.059	-2.890	-2.908	-2.832
18	0.061	0.057	0.062	-2.792	-2.870	-2.777
22	0.070	0.081	0.081	-2.662	-2.519	-2.519
25	0.081	0.095	0.090	-2.511	-2.358	-2.408
28	0.102	0.100	0.117	-2.280	-2.308	-2.150
31	0.179	0.156	0.200	-1.719	-1.855	-1.611
34	0.322	0.346	0.365	-1.132	-1.062	-1.007
39	0.547	0.731	0.680	-0.604	-0.313	-0.386
42	0.654	0.780	0.735	-0.424	-0.249	-0.307
45	0.754	0.912	0.771	-0.282	-0.092	-0.260
48	0.850	1.002	0.865	-0.163	0.002	-0.145
51	0.946	1.214	0.897	-0.055	0.194	-0.108
54	1.064	1.223	0.902	0.062	0.202	-0.103
60	1.113	1.165	0.911	0.107	0.153	-0.093
64	1.005	1.009	0.897	0.005	0.009	-0.108



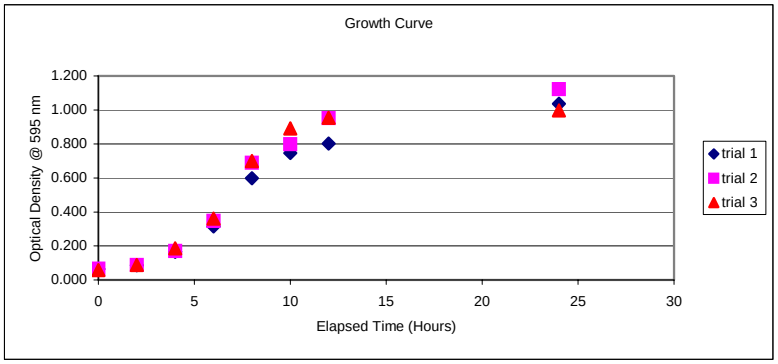
	Growth Rate
trial 1	0.1528
tiral 2	0.1865
trial 3	0.1613
average	0.1669
std	0.0175



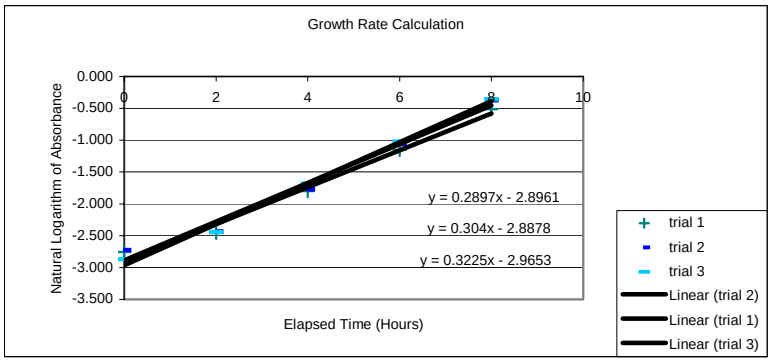
Both

60 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.063	0.066	0.057	-2.760	-2.726	-2.872
2	0.086	0.088	0.087	-2.458	-2.432	-2.442
4	0.165	0.170	0.186	-1.799	-1.771	-1.685
6	0.315	0.347	0.361	-1.154	-1.060	-1.020
8	0.598	0.689	0.700	-0.515	-0.372	-0.357
10	0.746	0.799	0.891	-0.293	-0.225	-0.115
12	0.802	0.952	0.955	-0.220	-0.050	-0.046
24	1.037	1.123	0.997	0.036	0.116	-0.003



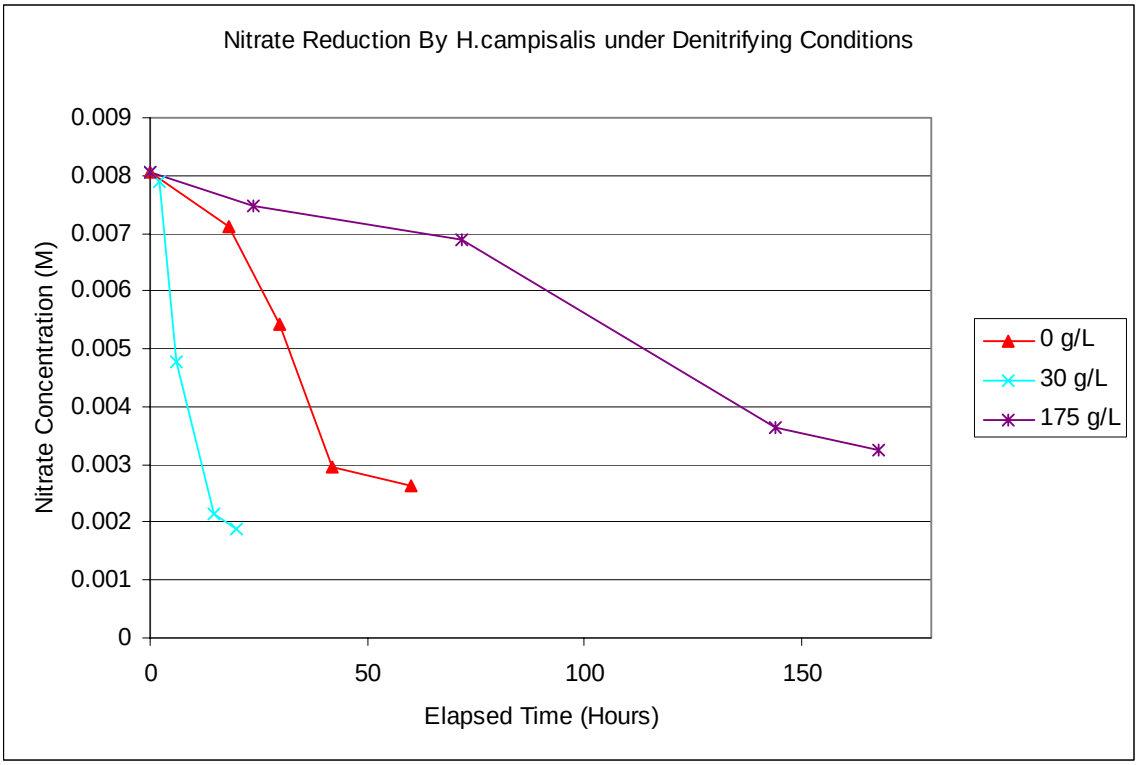
	Growth Rate
trial 1	0.2897
tiral 2	0.3040
trial 3	0.3225
average	0.3054
std	0.0164



Appendix H

Appendix H depicts the ability of *H. campisalis* to use nitrate as an electron acceptor. A colorimetric kit was used to quantify changing levels of nitrate in the sample.

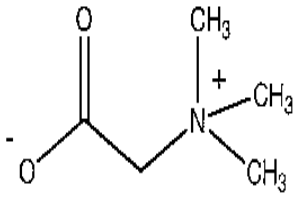
Nitrate reduction by *H. campisalis*



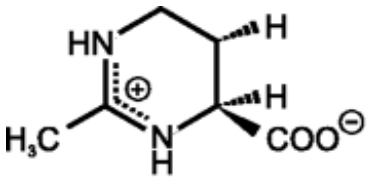
Appendix I

Appendix I illustrates the chemical structures of the three organic compatible solutes identified in *H. campisalis*. Of note is the similarity between ectoine and hydroxyectoine. The additional hydroxyl group present on hydroxyectoine increases the effectiveness of hydroxyectoine as a osmotic protectant. The simple linear nature of glycine betaine may be the reason that cells commonly uptake it from the environment.

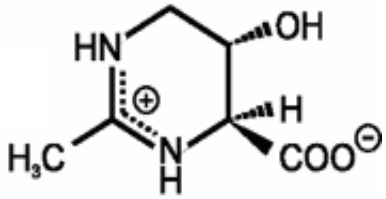
Chemical structures for compatible solutes



Glycine Betaine



Ectoine



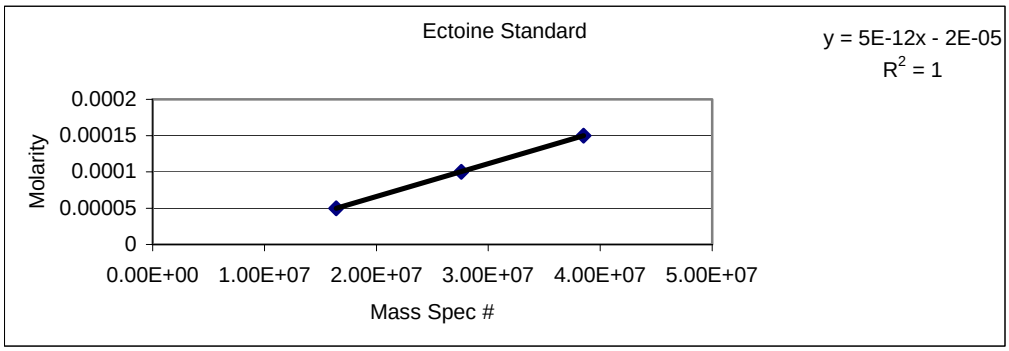
Hydroxyectoine

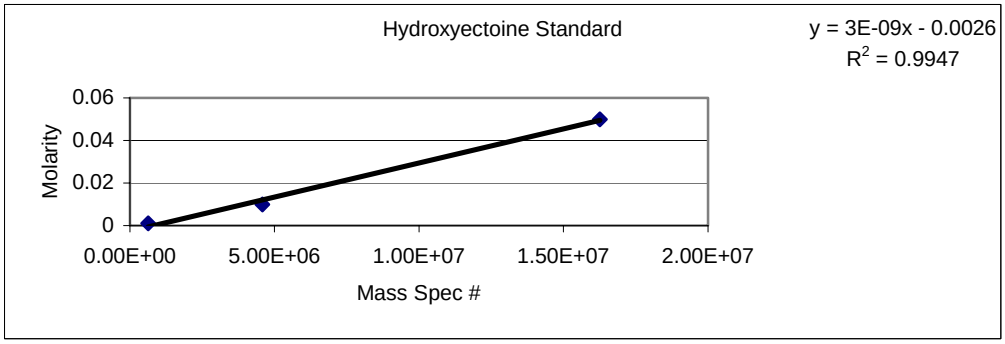
Appendix J

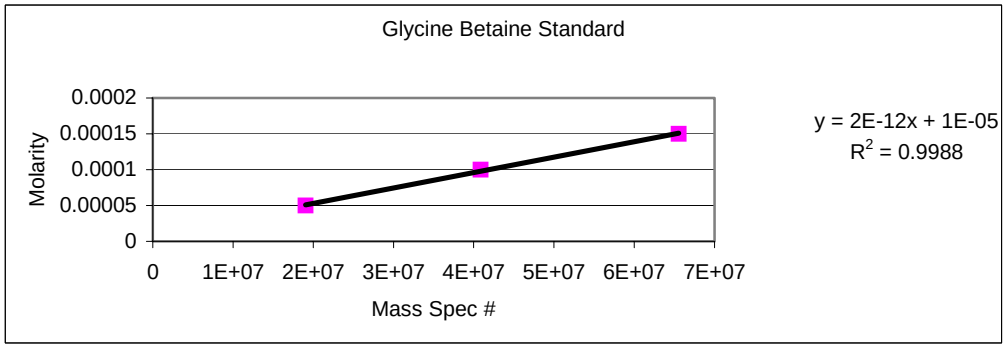
In order to quantify the compatible solutes identified in *H. campisalis*, it was necessary to create standards to generate a correlation between the peak intensity measured by the mass spectrometry and the concentration of compatible solute in the extraction mixture. These standards are illustrated in appendix J. The correlations are presented on the respective graphs.

Compatible solute standards

Ectoine		Glycine Betaine	Hydroxyectoine	
Concentration (M)	Intensity	Intensity	Concentration (M)	Intensity
0.00005	16420352	19058688	0.001	631206
0.00010	27577344	40878080	0.01	4586752
0.00015	38510592	65536000	0.05	16257024







Appendix K

Appendix K contains a table of the compatible solute accumulation observed in *H. campisalis* in aerobic and denitrifying conditions as a function of salinity. Using the correlations presented in appendix J, the molarity compatible solute of interest can be determined within the extraction mixture. Compatible solutes within the extraction mixture were extracted from a known dry weight of cell material. Cell count data collected earlier from a separate analysis provided a cell density approximation. The approximate geometry of *H. campisalis* was used to calculate a cell volume of 7.854×10^{-16} Liters. Given the cellular weight, cellular volume, and mole compatible solute/cell gram a cellular molarity was calculated.

Cytoplasmic compatible solute accumulation (mM)

Hydroxyectoine

NaCl g/L (Aerobic)	Trial 1	Trial 2	Trial 3	AVERAGE	STD
0	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00	0.00
175	26.69	35.11	19.01	26.94	8.05
NaCl g/L (Denitrifying)					
0	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00	0.00
175	41.48	26.51	32.87	33.62	7.51

Ectoine

NaCl g/L (Aerobic)	Trial 1	Trial 2	Trial 3	AVERAGE	STD
0	56.33	75.65	77.91	69.96	11.86
20	119.61	138.77	103.39	120.59	17.71
30	206.07	223.87	184.45	204.80	19.74
90	475.12	521.17	511.60	502.63	24.30
175	262.73	368.19	326.96	319.29	53.14
NaCl g/L (Denitrifying)					
0	57.17	30.20	57.77	48.38	15.75
20	38.07	49.68	25.40	37.72	12.14
30	80.12	131.69	150.52	120.78	36.45
90	263.79	323.12	264.80	283.90	33.97
175	71.97	68.29	104.59	81.62	19.98

Glycine Betaine

NaCl g/L (Aerobic)	Trial 1	Trial 2	Trial 3	AVERAGE	STD
0	13.63	15.53	33.16	20.77	10.77
20	57.17	82.49	79.12	72.93	13.75
30	65.17	44.37	49.95	53.16	10.77
90	0.00	0.00	0.00	0.00	0.00
175	0.00	0.00	0.00	0.00	0.00
NaCl g/L (Denitrifying)					
0	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00
90	58.21	70.20	38.32	55.58	16.10
175	0.00	0.00	0.00	0.00	0.00

Appendix L

The cellular molarity of accumulated compatible solutes is not representative of the total compatible solute data. The reason for this is that the cell density is not constant over a range of salinities. The overall compatible solute productions was calculated and expressed as a environmental molarity. In this case, the environment was limited to the growth medium. To calculate this the molarity of the extraction mixture was simply extrapolated to the volume of the growth medium from which the extraction was performed.

Accumulation of compatible solutes on medium basis (mM)

Hydroxyectoine

NaCl g/L (Aerobic)	Trial 1	Trial 2	Trial 3	AVERAGE	STD
0	0.0000	0.0000	0.0000	0.0000	0.0000
20	0.0000	0.0000	0.0000	0.0000	0.0000
30	0.0000	0.0000	0.0000	0.0000	0.0000
90	0.0000	0.0000	0.0000	0.0000	0.0000
175	0.0028	0.0085	0.0051	0.0054	0.0029
NaCl g/L (Denitrifying)					
0	0.0000	0.0000	0.0000	0.0000	0.0000
20	0.0000	0.0000	0.0000	0.0000	0.0000
30	0.0000	0.0000	0.0000	0.0000	0.0000
90	0.0000	0.0000	0.0000	0.0000	0.0000
175	0.0065	0.0056	0.0077	0.0066	0.0011

Ectoine

NaCl g/L (Aerobic)	Trial 1	Trial 2	Trial 3	AVERAGE	STD
0	0.0133	0.0127	0.0161	0.0140	0.0018
20	0.0659	0.0432	0.0414	0.0502	0.0137
30	0.1243	0.1226	0.0958	0.1143	0.0160
90	0.1243	0.1492	0.1682	0.1472	0.0220
175	0.0275	0.0889	0.0871	0.0678	0.0349
NaCl g/L (Denitrifying)					
0	0.0133	0.0123	0.0227	0.0161	0.0057
20	0.0137	0.0317	0.0124	0.0193	0.0108
30	0.0344	0.0551	0.0567	0.0487	0.0125
90	0.0764	0.0423	0.0337	0.0508	0.0226
175	0.0112	0.0144	0.0245	0.0167	0.0070

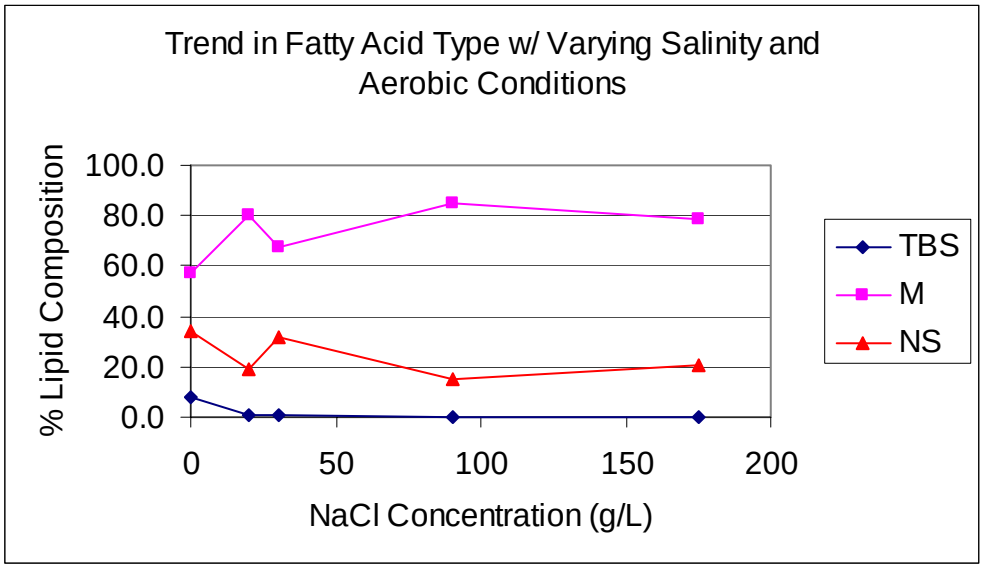
Glycine Betaine

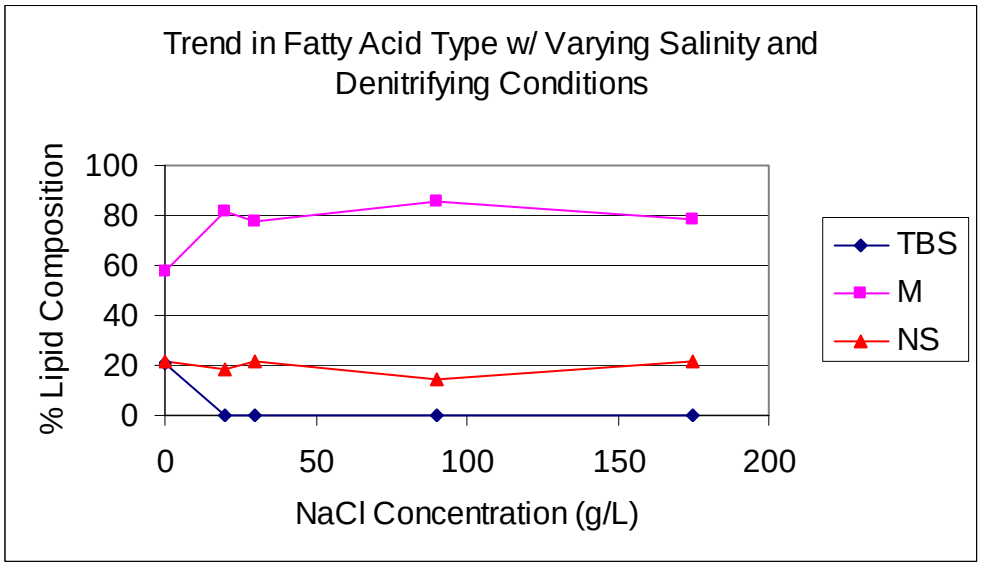
NaCl g/L (Aerobic)	Trial 1	Trial 2	Trial 3	AVERAGE	STD
0	0.0032	0.0026	0.0068	0.0042	0.0023
20	0.0315	0.0257	0.0317	0.0296	0.0034
30	0.0393	0.0243	0.0259	0.0299	0.0082
90	0.0000	0.0000	0.0000	0.0000	0.0000
175	0.0000	0.0000	0.0000	0.0000	0.0000
NaCl g/L (Denitrifying)					
0	0.0000	0.0000	0.0000	0.0000	0.0000
20	0.0000	0.0000	0.0000	0.0000	0.0000
30	0.0000	0.0000	0.0000	0.0000	0.0000
90	0.0169	0.0092	0.0049	0.0103	0.0061
175	0.0000	0.0000	0.0000	0.0000	0.0000

Appendix M

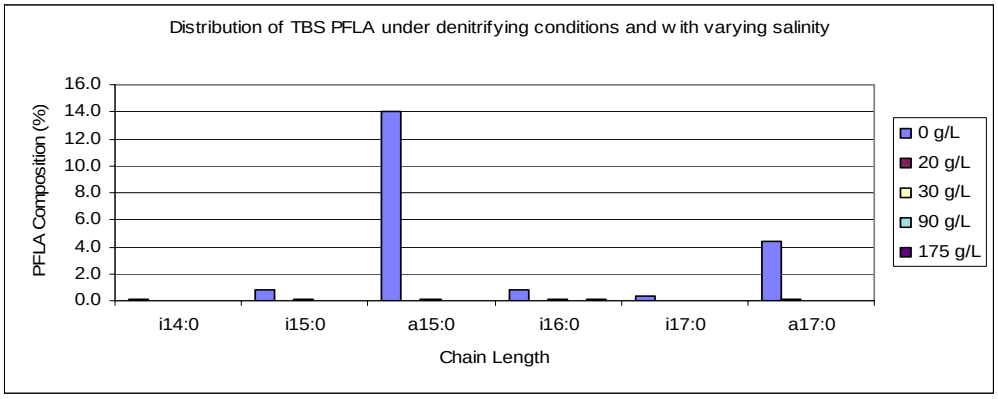
Appendix M presents all data collected in the PLFA analysis performed by Microbial Insights (Rockford, Tennessee) with *H. campisalis* samples. Three primary types of fatty acids were observed: terminally branched saturates (tbs), monoenoic acids, and natural saturates (ns). Among these three types the analysis was further broken up into chain length and transformation (cis, trans, and cyclic). The amount of trans and cyclic fatty acid conformations represent physiological markers, indicative of certain environmental stresses. The ratio of cyclic fatty acids to cis fatty acid transformations is accomplished by looking at the primary sub group of fatty acids. The predominant cyclic formation (either of 17 or 19 carbons) is compared to that of the predominant straight chain (typically a cis formation monoenoic acid). The ratio of trans to cis formations was done identically. Experiments were not repeated, however methods used by Microbial Insights typically have approximately 5% variance at the greatest.

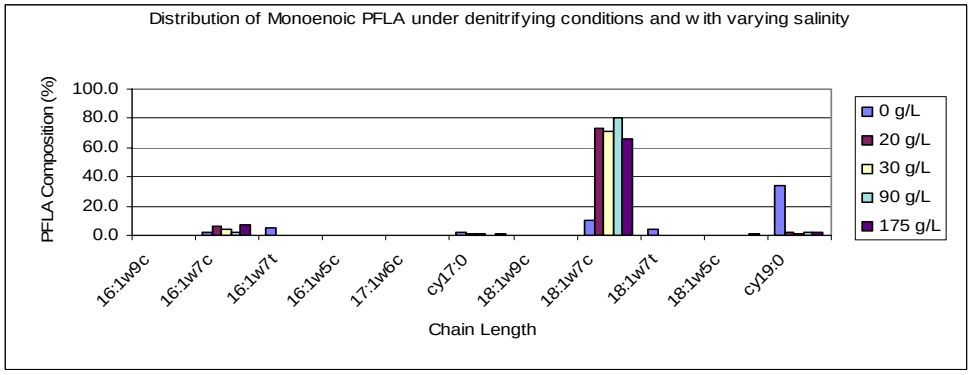
PFLA analysis data

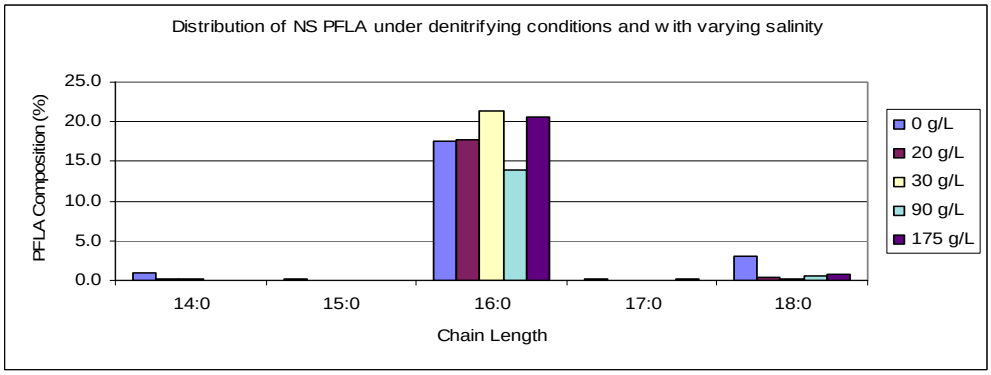




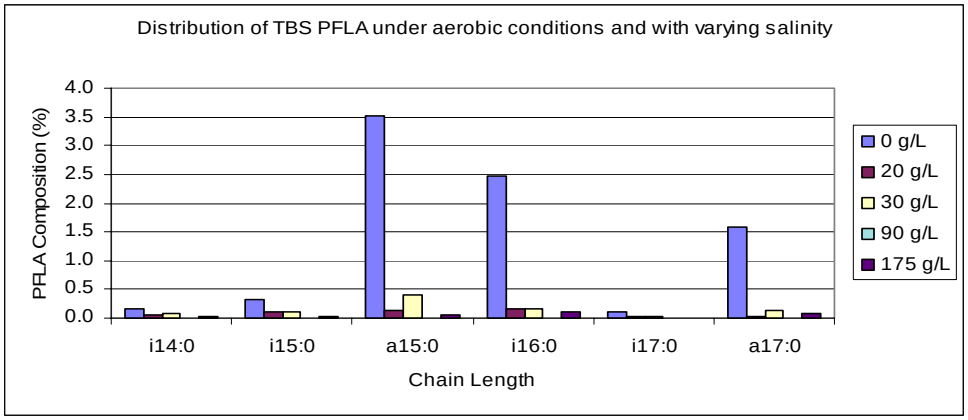
Complete PFLA composition under varying salinities and denitrifying conditions

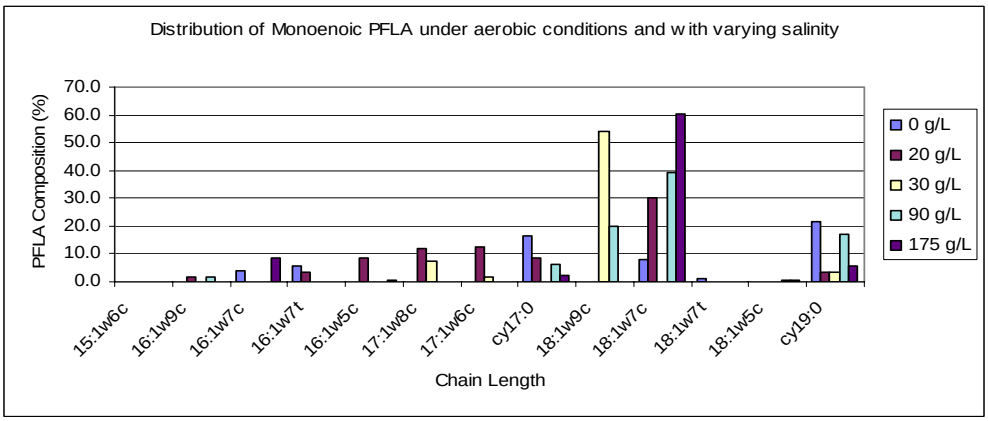


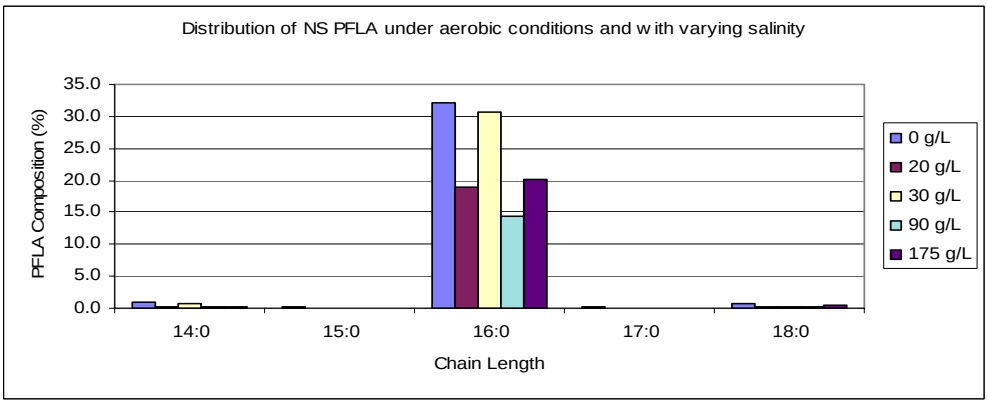




Complete PFLA composition under varying salinities and aerobic conditions







Appendix N

A brief protein analysis was done on *H. campisalis* under varying conditions by the protein sequencing lab at Purdue University by Dr. Doris Terry. 2-d gel electrophoresis was used to separate individual proteins and determine if salinity effected protein expression. The molecular weigh ladder in kDa goes as.

250

150

100

75

50

37

25

20

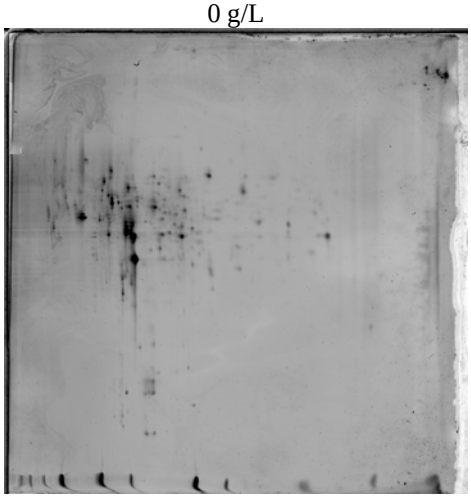
15

10

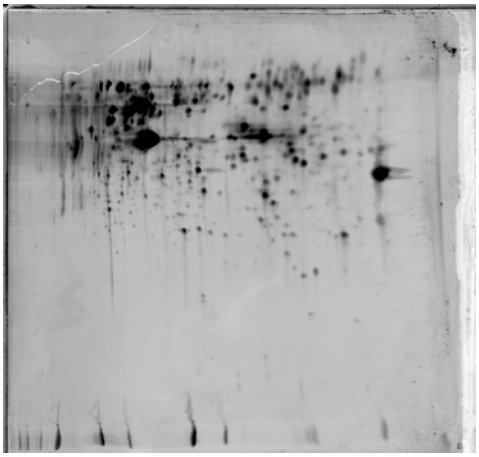
2-D gel protein analysis for *H. campisalis* with varying salinity

Aerobic

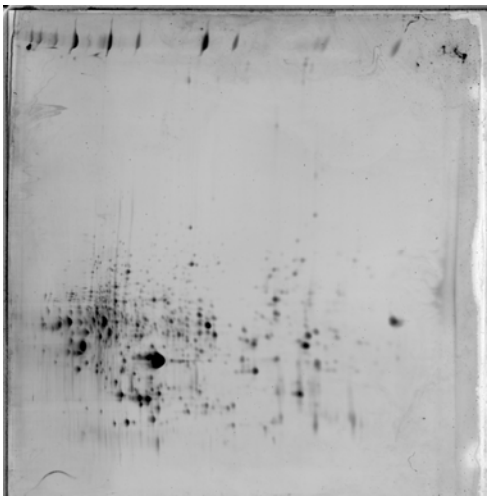
0 g/L



90 g/L

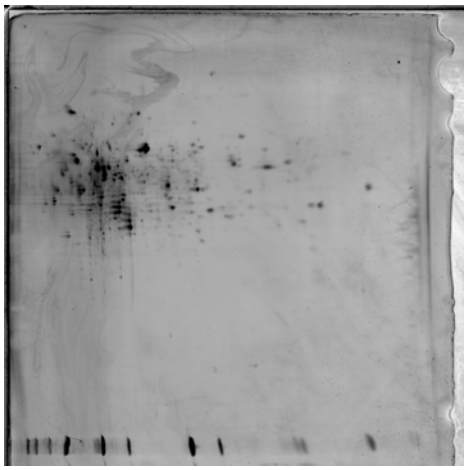


175 g/L

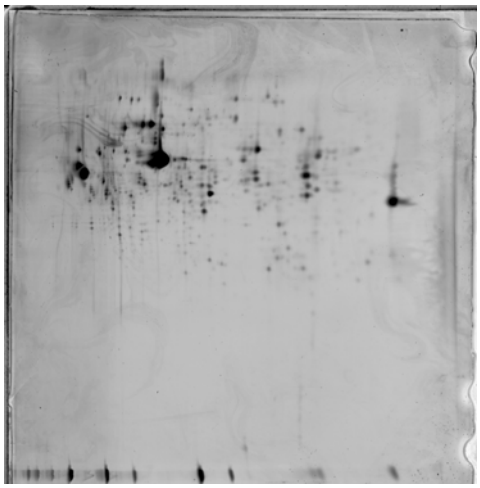


Denitrifying

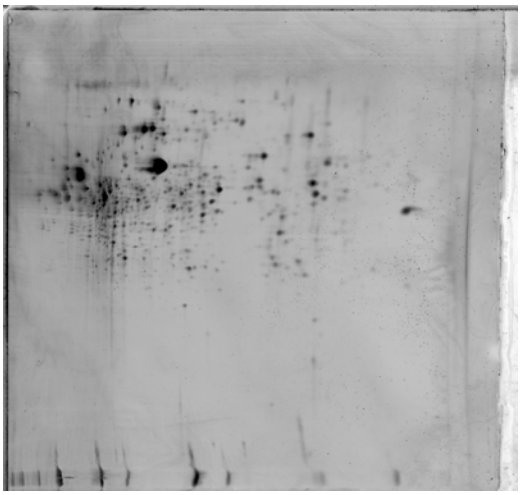
0 g/L



90 g/L



175 g/L

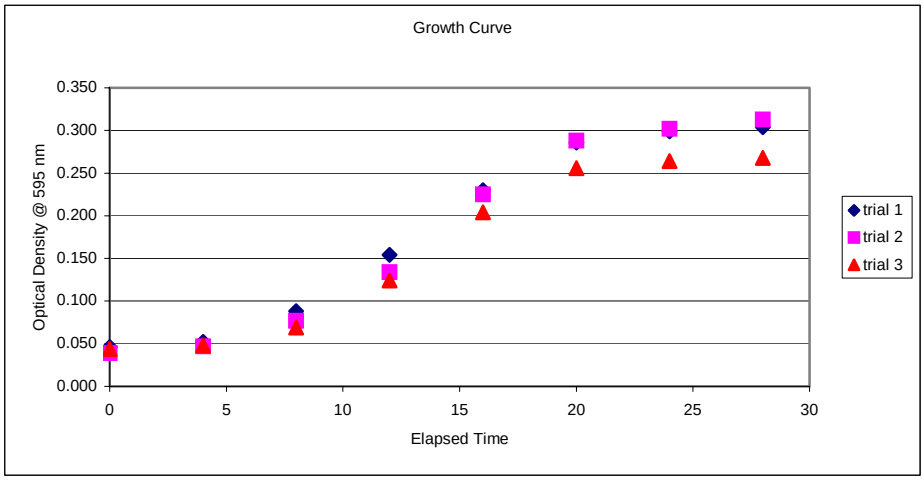


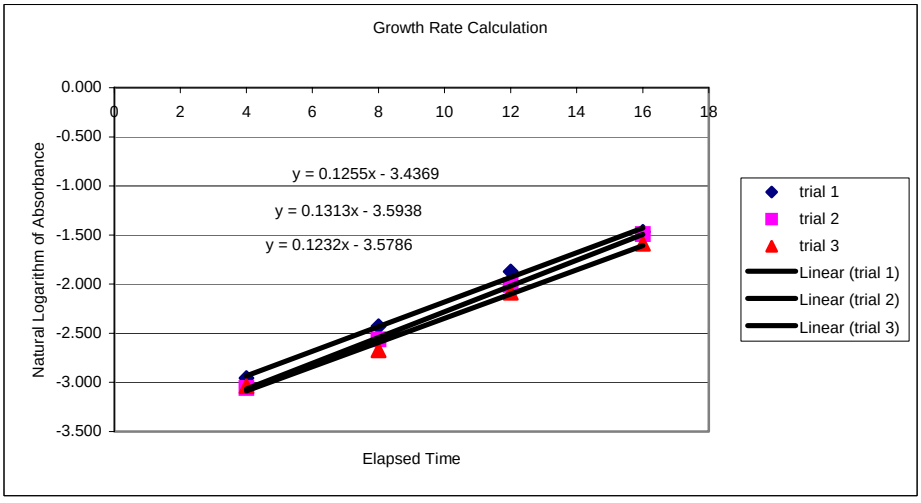
Appendix O

Appendix O contains data obtained in experiments designed to determine the Monod parameters K_s and μ_{max} under varying salinities. μ_{max} were determined experimentally along with μ. The following relation: $\mu = \frac{\mu_{max} C_s}{C_s + K_s}$ was used to solve for a K_s value using a Microsoft excel solver package. Experiments were repeated in triplicate with individual K_s values being determined for each sample to determine statistical significance. The resulting Monod parameters were used to approximate specific growth rates and these were in turn compared with actual values to determine the significance of substrate level in the growth of *H. campisalis* with increasing salinity. The conditions at which the data was collected on the following pages are denoted in the upper left corner with the salinity being represented outside of the parenthesis, and the substrate concentrations being represented within the parenthesis in g/L. Data from previous experiments at 30 and 90 g/L NaCl was used at 1 g/L glucose (Appendix D).

30 g (0.1)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.046	0.039	0.044	-3.079	-3.244	-3.124
4	0.052	0.047	0.048	-2.957	-3.058	-3.037
8	0.088	0.077	0.069	-2.430	-2.564	-2.674
12	0.154	0.134	0.124	-1.871	-2.010	-2.087
16	0.230	0.225	0.204	-1.470	-1.492	-1.590
20	0.286	0.288	0.256	-1.252	-1.245	-1.363
24	0.299	0.302	0.264	-1.207	-1.197	-1.332
28	0.304	0.313	0.268	-1.191	-1.162	-1.317

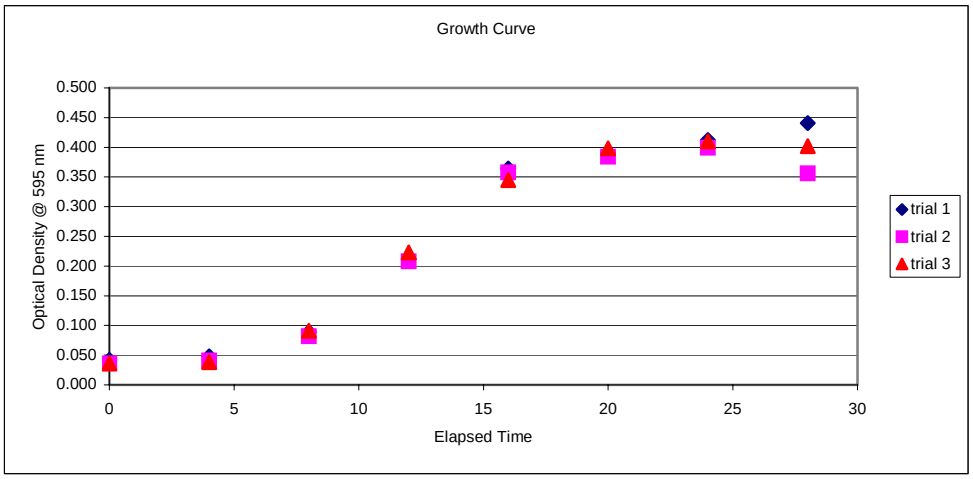


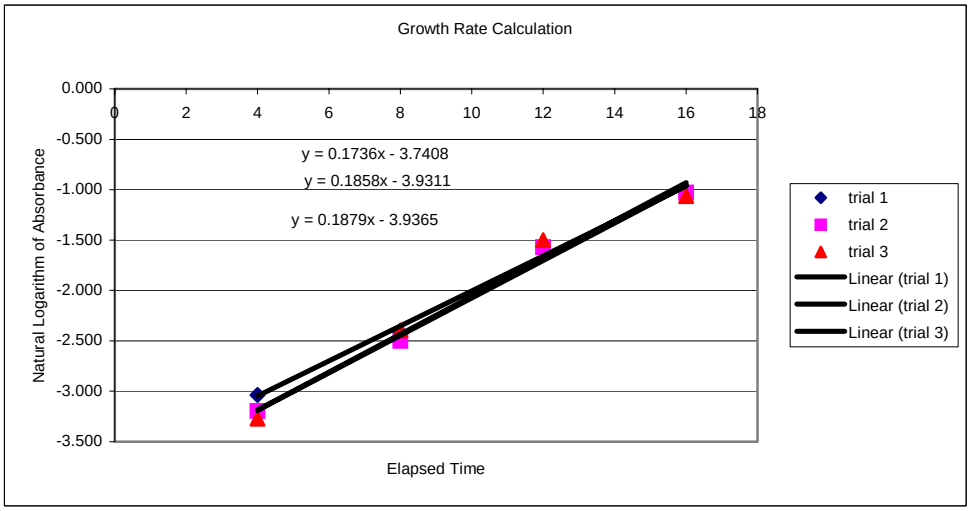


trial 1	trial 2	trial 3	Growth Rate	STD
0.1255	0.1313	0.1232	0.1267	0.0042

30 g (0.25)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.042	0.036	0.036	-3.170	-3.324	-3.324
4	0.048	0.041	0.038	-3.037	-3.194	-3.270
8	0.089	0.082	0.091	-2.419	-2.501	-2.397
12	0.212	0.208	0.223	-1.551	-1.570	-1.501
16	0.364	0.358	0.345	-1.011	-1.027	-1.064
20	0.391	0.384	0.398	-0.939	-0.957	-0.921
24	0.412	0.399	0.410	-0.887	-0.919	-0.892
28	0.441	0.356	0.402	-0.819	-1.033	-0.911

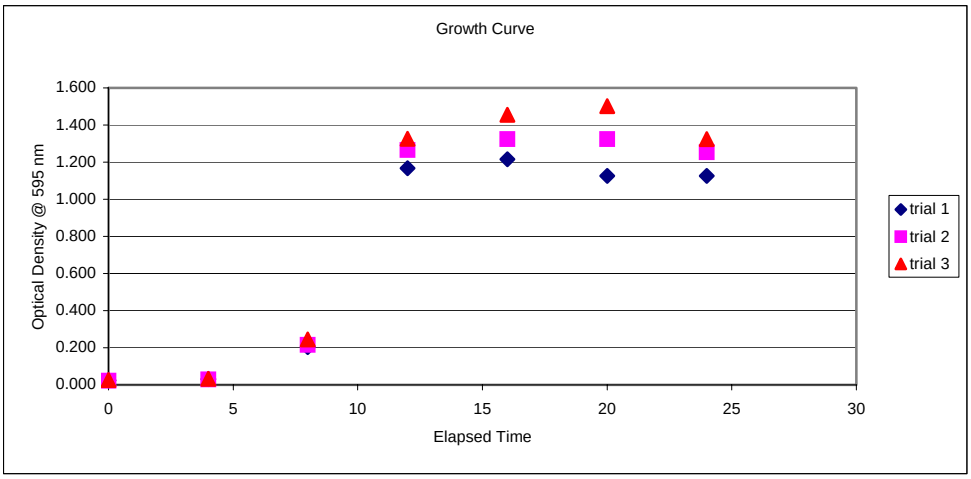


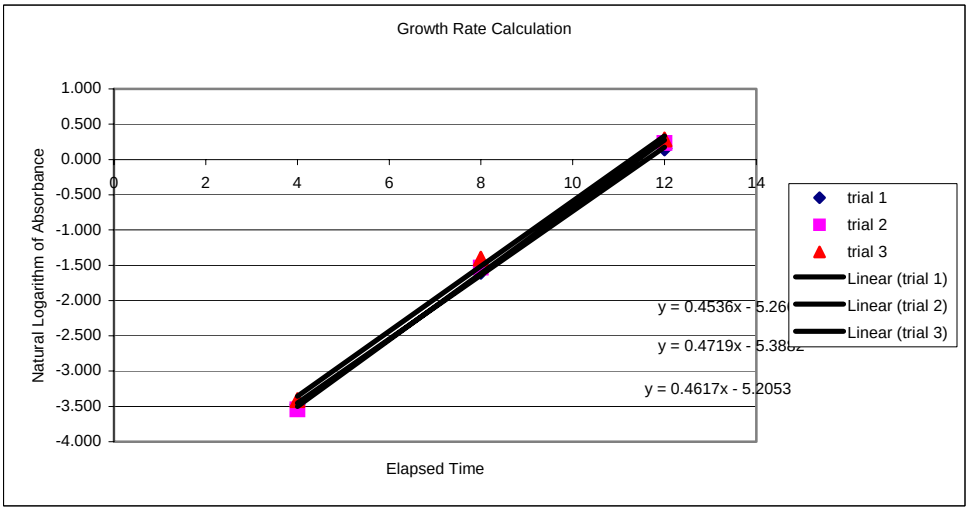


trial 1	trial 2	trial 3	Growth Rate	STD
0.1736	0.1858	0.1879	0.1824	0.0077

30 g (2)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.025	0.022	0.026	-3.689	-3.817	-3.650
4	0.031	0.029	0.033	-3.474	-3.540	-3.411
8	0.203	0.216	0.245	-1.595	-1.532	-1.406
12	1.168	1.265	1.326	0.155	0.235	0.282
16	1.216	1.325	1.456	0.196	0.281	0.376
20	1.126	1.324	1.502	0.119	0.281	0.407
24	1.126	1.253	1.325	0.119	0.226	0.281

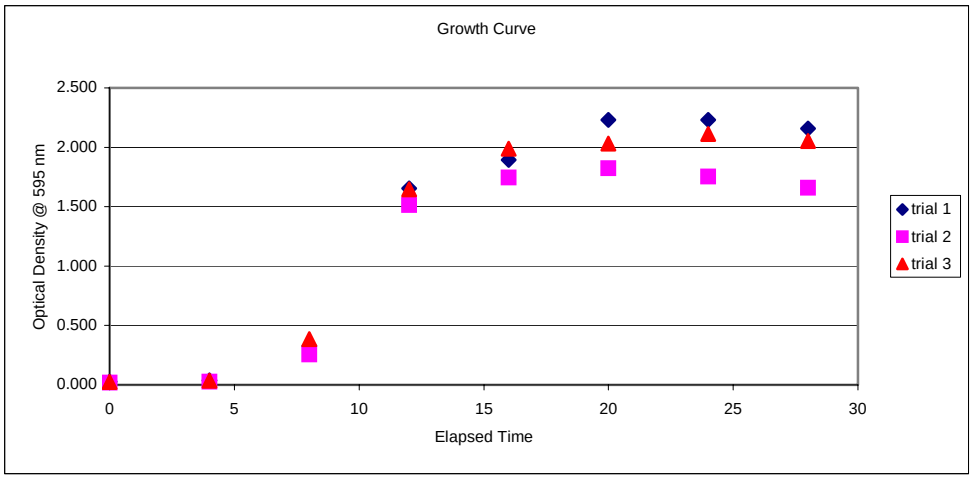


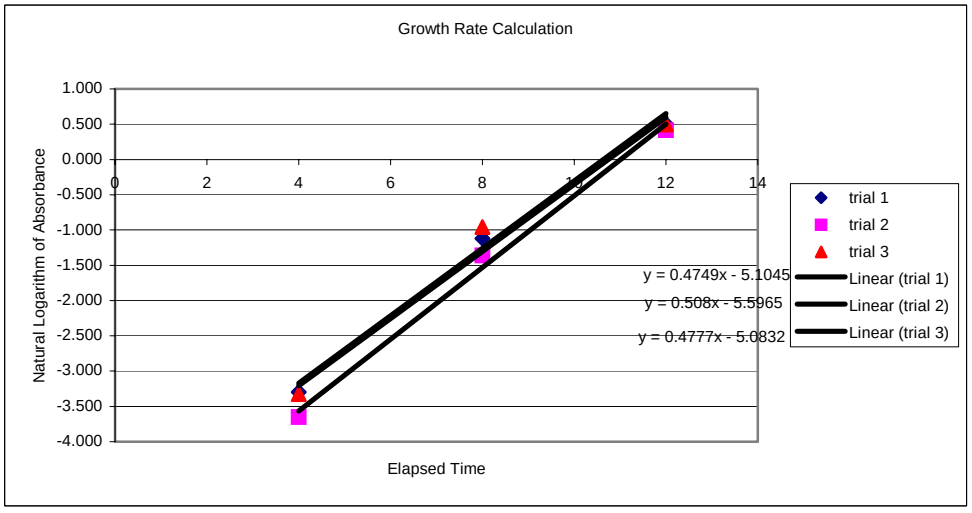


trial 1	trial 2	trial 3	Growth Rate	STD
0.4536	0.4719	0.4617	0.4624	0.0092

30 g (5)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.026	0.019	0.025	-3.650	-3.963	-3.689
4	0.037	0.026	0.036	-3.297	-3.650	-3.324
8	0.326	0.256	0.384	-1.121	-1.363	-0.957
12	1.653	1.513	1.645	0.503	0.414	0.498
16	1.893	1.746	1.987	0.638	0.557	0.687
20	2.231	1.824	2.031	0.802	0.601	0.709
24	2.231	1.754	2.113	0.802	0.562	0.748
28	2.158	1.659	2.054	0.769	0.506	0.720

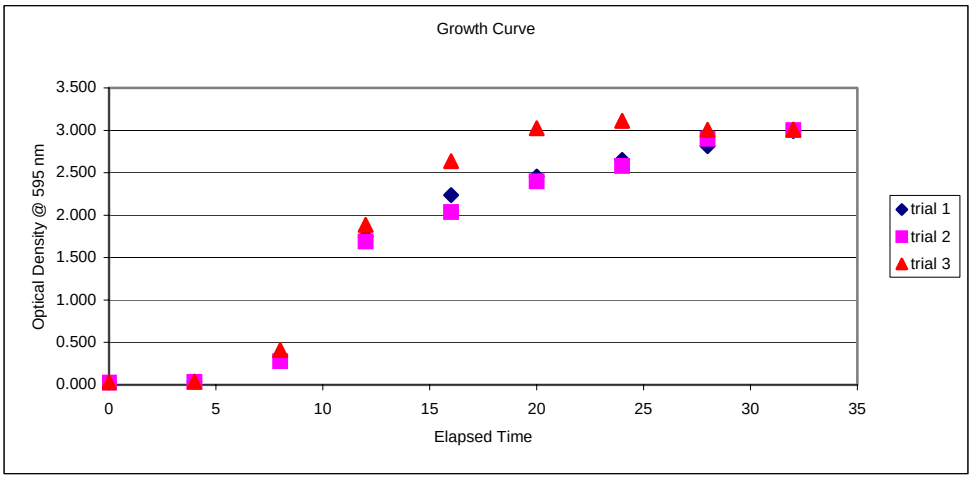


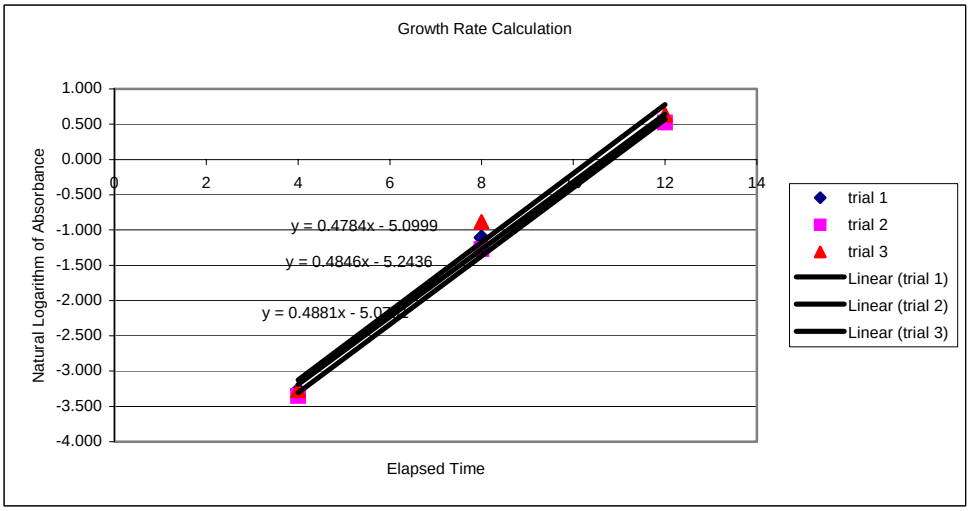


trial 1	trial 2	trial 3	Growth Rate	STD
0.4749	0.5080	0.4777	0.4869	0.0184

30 g (10)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.031	0.028	0.032	-3.474	-3.576	-3.442
4	0.038	0.035	0.038	-3.270	-3.352	-3.270
8	0.331	0.280	0.412	-1.106	-1.273	-0.887
12	1.745	1.689	1.887	0.557	0.524	0.635
16	2.235	2.035	2.635	0.804	0.710	0.969
20	2.456	2.398	3.023	0.899	0.875	1.106
24	2.651	2.581	3.112	0.975	0.948	1.135
28	2.812	2.900	3.005	1.034	1.065	1.100
32	2.991	3.002	3.010	1.096	1.099	1.102

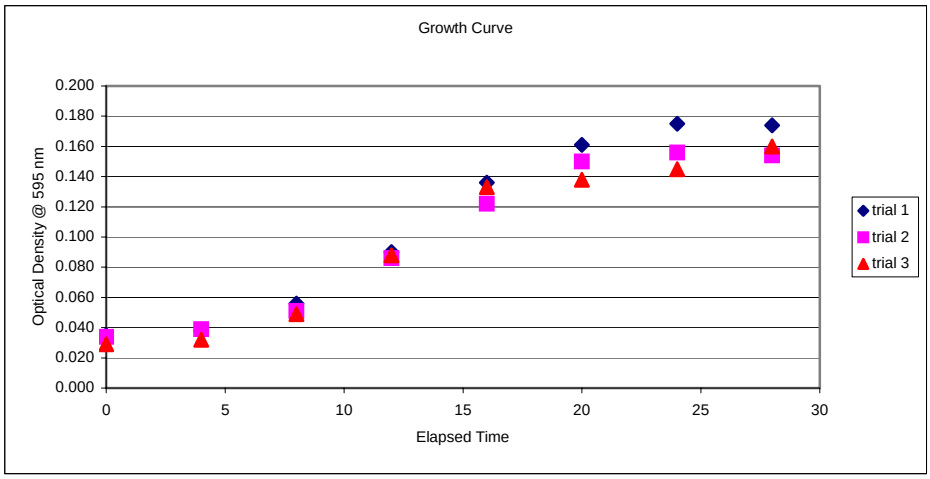


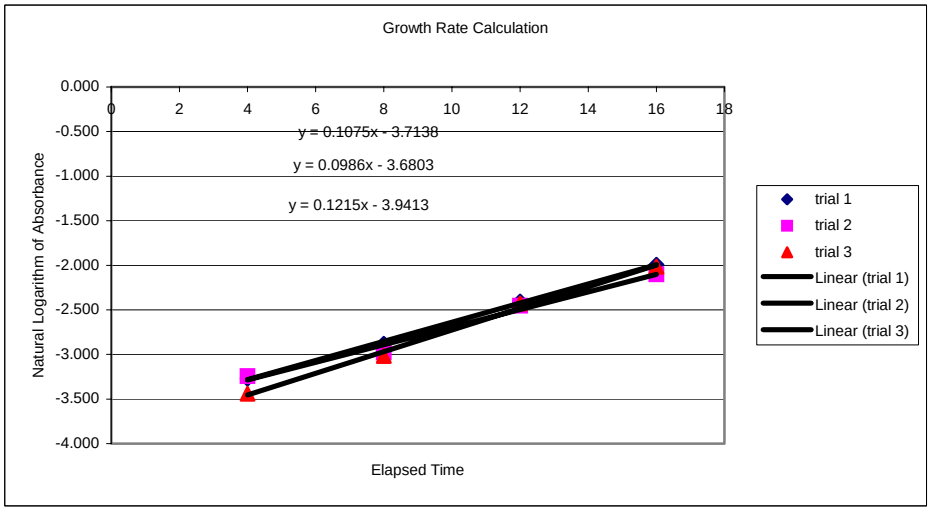


trial 1	trial 2	trial 3	Growth Rate	STD
0.4784	0.4846	0.4881	0.4837	0.0049

50 g (0.1)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.035	0.034	0.029	-3.352	-3.381	-3.540
4	0.038	0.039	0.032	-3.270	-3.244	-3.442
8	0.056	0.051	0.049	-2.882	-2.976	-3.016
12	0.090	0.086	0.088	-2.408	-2.453	-2.430
16	0.136	0.122	0.133	-1.995	-2.104	-2.017
20	0.161	0.150	0.138	-1.826	-1.897	-1.981
24	0.175	0.156	0.145	-1.743	-1.858	-1.931
28	0.174	0.154	0.160	-1.749	-1.871	-1.833

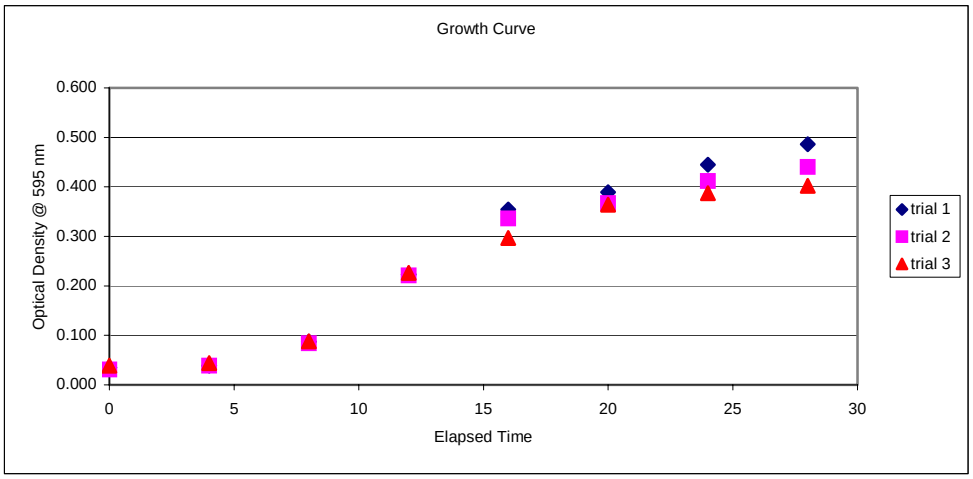


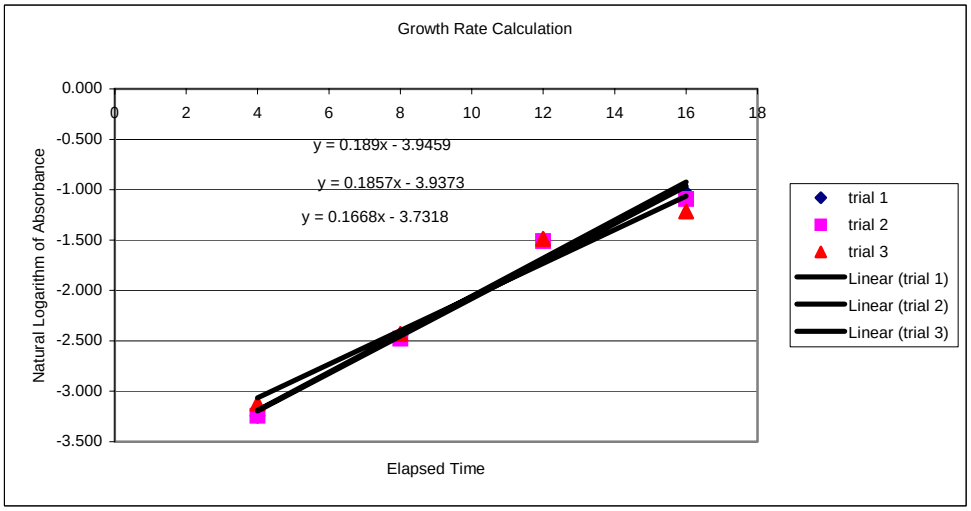


trial 1	trial 2	trial 3	Growth Rate	STD
0.1075	0.0986	0.1215	0.1092	0.0115

50 g (0.25)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.035	0.031	0.039	-3.352	-3.474	-3.244
4	0.039	0.039	0.044	-3.244	-3.244	-3.124
8	0.087	0.084	0.088	-2.442	-2.477	-2.430
12	0.223	0.221	0.226	-1.501	-1.510	-1.487
16	0.354	0.336	0.297	-1.038	-1.091	-1.214
20	0.389	0.367	0.364	-0.944	-1.002	-1.011
24	0.445	0.412	0.387	-0.810	-0.887	-0.949
28	0.486	0.440	0.402	-0.722	-0.821	-0.911

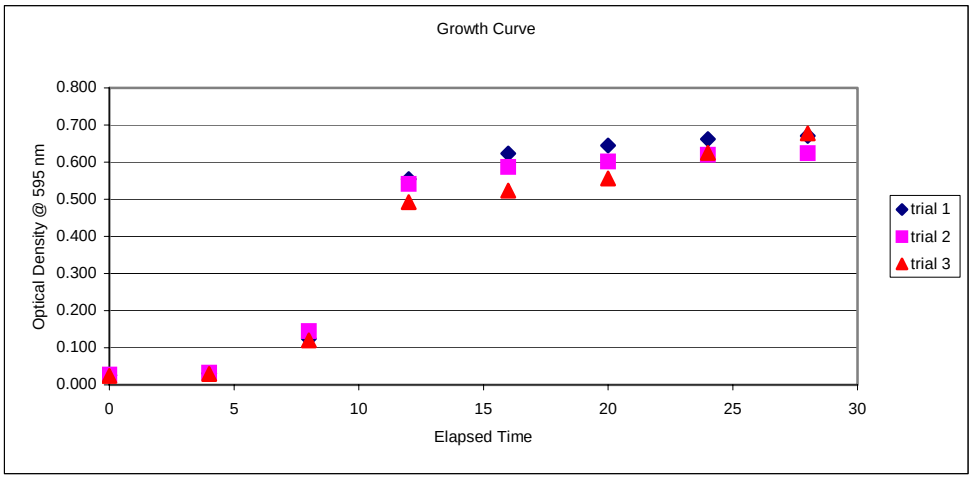


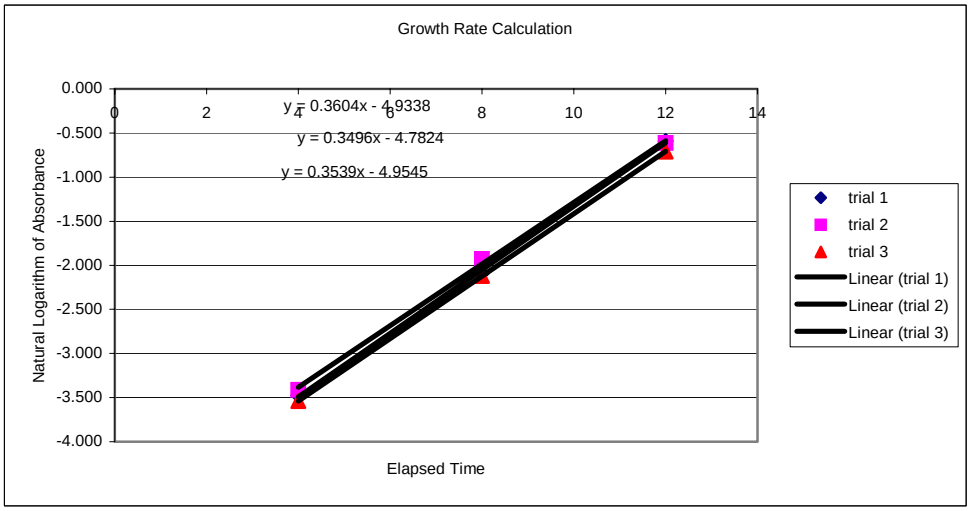


trial 1	trial 2	trial 3	Growth Rate	STD
0.1890	0.1857	0.1668	0.1805	0.0120

50 g (1)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.026	0.028	0.024	-3.650	-3.576	-3.730
4	0.031	0.033	0.029	-3.474	-3.411	-3.540
8	0.124	0.145	0.120	-2.087	-1.931	-2.120
12	0.554	0.541	0.492	-0.591	-0.614	-0.709
16	0.623	0.587	0.523	-0.473	-0.533	-0.648
20	0.645	0.602	0.556	-0.439	-0.507	-0.587
24	0.662	0.620	0.625	-0.412	-0.478	-0.470
28	0.671	0.624	0.678	-0.399	-0.472	-0.389

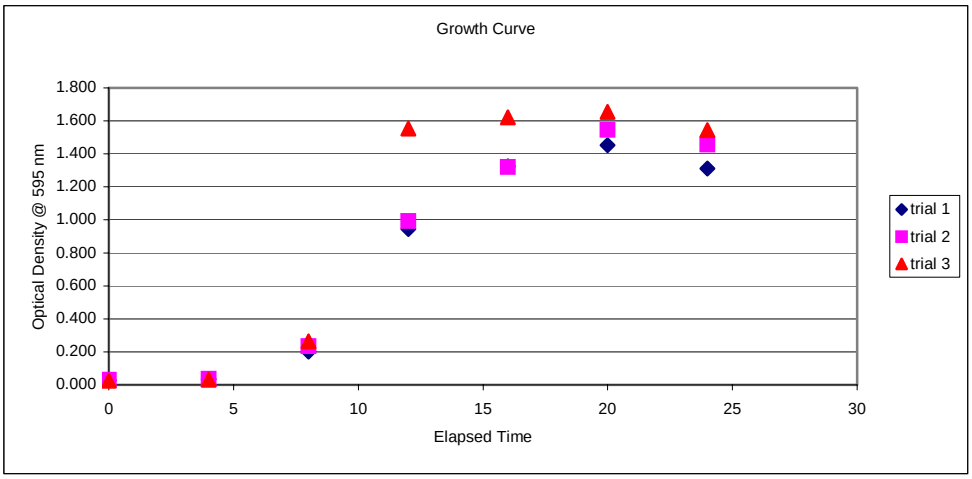


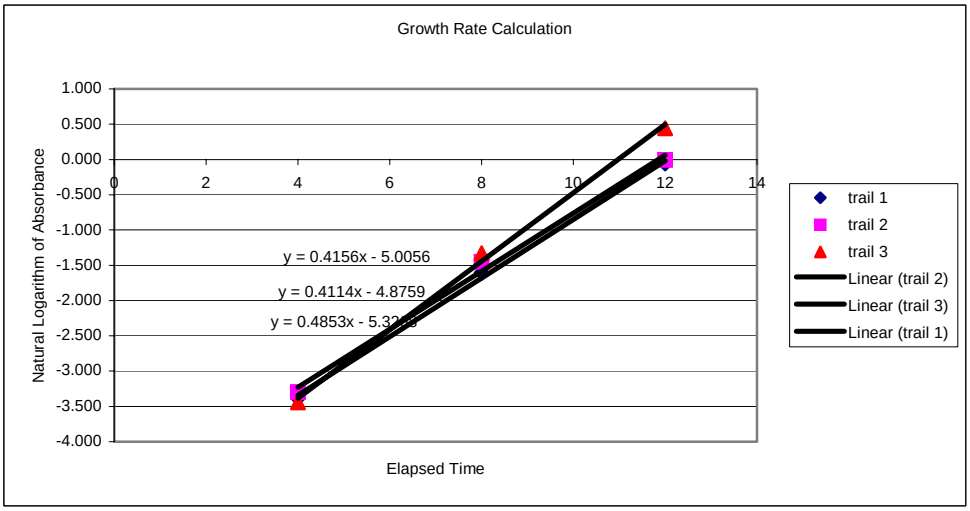


trial 1	trial 2	trial 3	Growth Rate	STD
0.3604	0.3496	0.3539	0.3546	0.0054

50 g (2)

elapsed time	trial 1	trial 2	trial 3	trail 1	trail 2	trail 3
0	0.028	0.032	0.025	-3.576	-3.442	-3.689
4	0.034	0.037	0.032	-3.381	-3.297	-3.442
8	0.201	0.234	0.264	-1.604	-1.452	-1.332
12	0.945	0.994	1.553	-0.057	-0.006	0.440
16	1.324	1.321	1.621	0.281	0.278	0.483
20	1.452	1.546	1.654	0.373	0.436	0.503
24	1.312	1.456	1.544	0.272	0.376	0.434

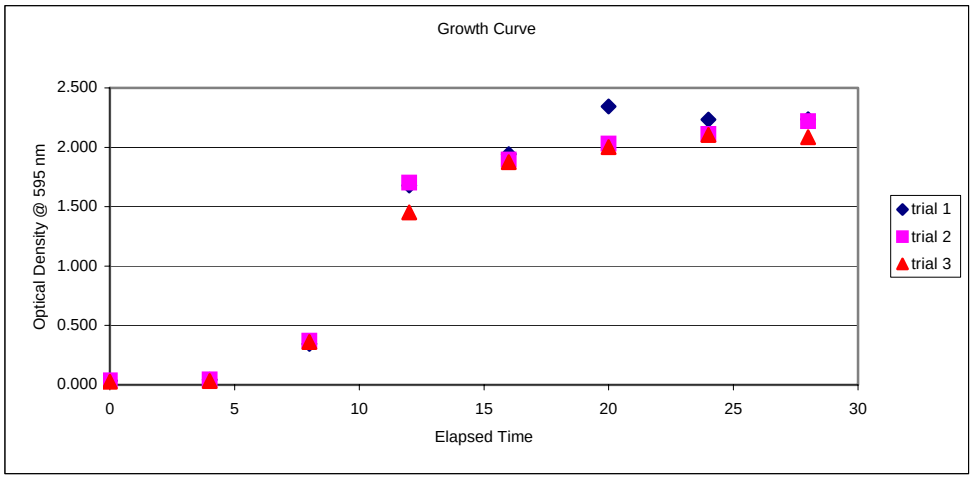


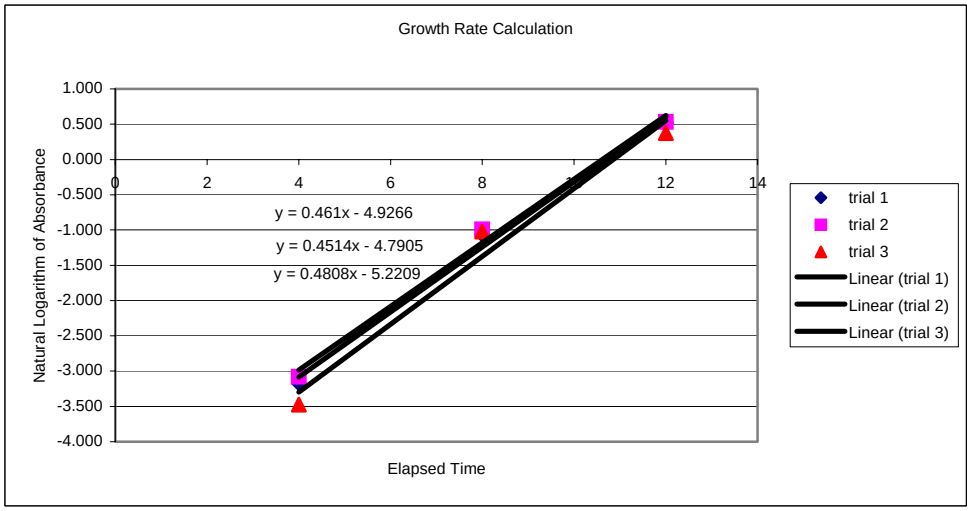


trial 1	trial 2	trial 3	Growth Rate	STD
0.4156	0.4114	0.4853	0.4374	0.0415

50 g (5)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.034	0.039	0.026	-3.381	-3.244	-3.650
4	0.042	0.046	0.031	-3.170	-3.079	-3.474
8	0.345	0.371	0.360	-1.064	-0.992	-1.022
12	1.678	1.702	1.452	0.518	0.532	0.373
16	1.942	1.897	1.874	0.664	0.640	0.628
20	2.344	2.031	2.001	0.852	0.709	0.694
24	2.234	2.113	2.105	0.804	0.748	0.744
28	2.236	2.221	2.086	0.805	0.798	0.735

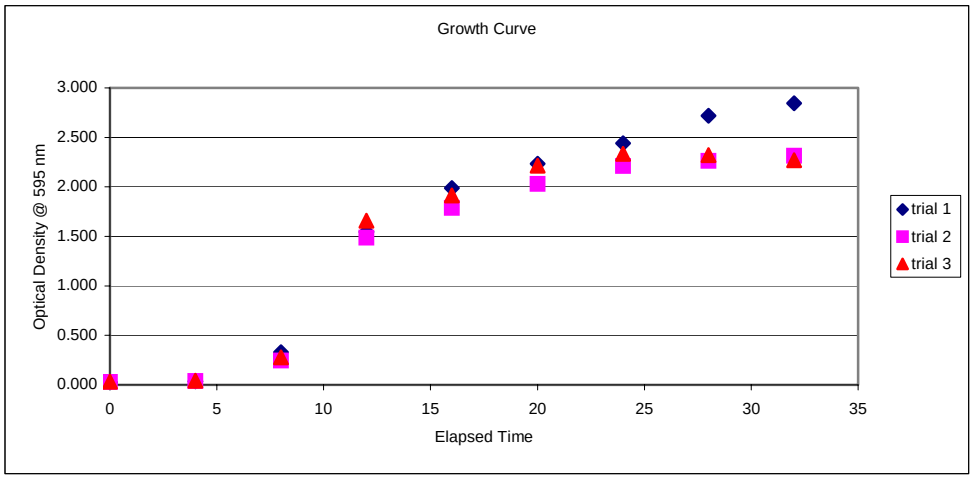


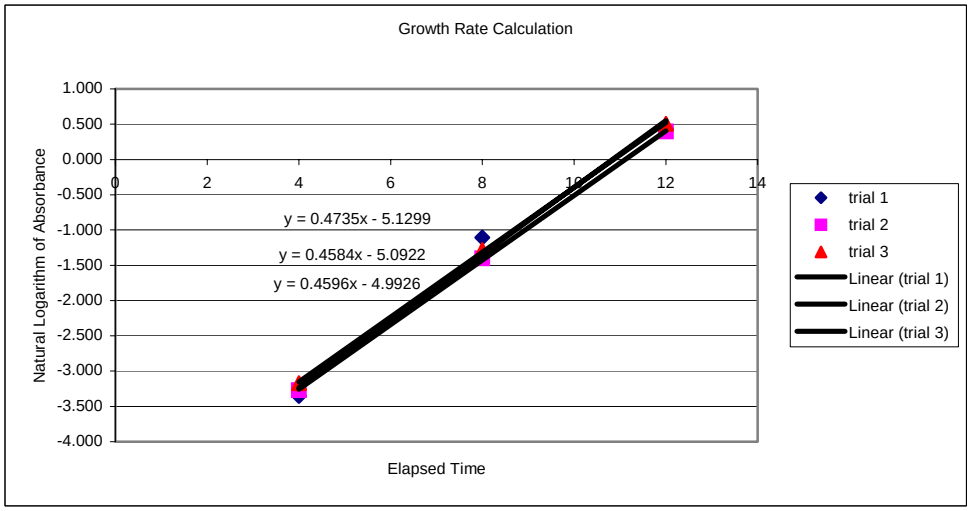


trial 1	trial 2	trial 3	Growth Rate	STD
0.4610	0.4514	0.4808	0.4644	0.0150

50 g (10)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.027	0.030	0.033	-3.612	-3.507	-3.411
4	0.035	0.038	0.042	-3.352	-3.270	-3.170
8	0.330	0.246	0.277	-1.109	-1.402	-1.284
12	1.546	1.487	1.660	0.436	0.397	0.507
16	1.987	1.789	1.913	0.687	0.582	0.649
20	2.234	2.031	2.214	0.804	0.709	0.795
24	2.441	2.210	2.334	0.892	0.793	0.848
28	2.720	2.264	2.321	1.001	0.817	0.842
32	2.845	2.314	2.270	1.046	0.839	0.820

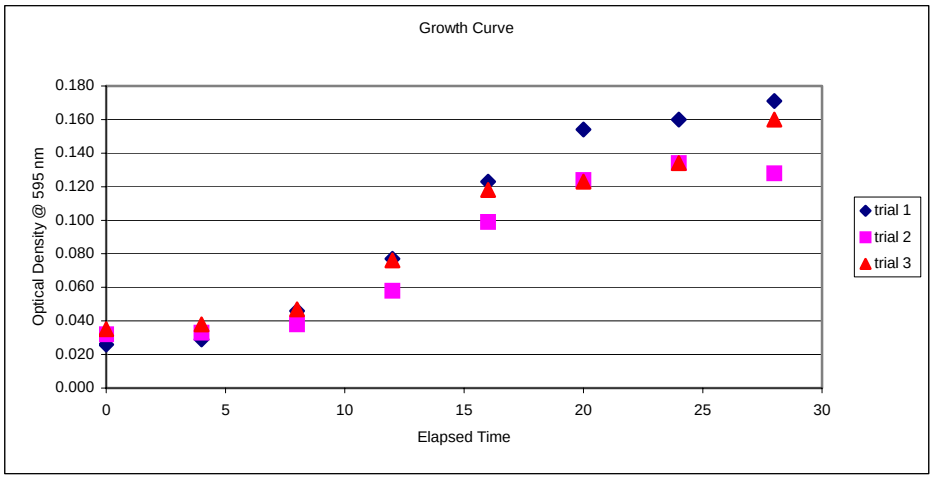


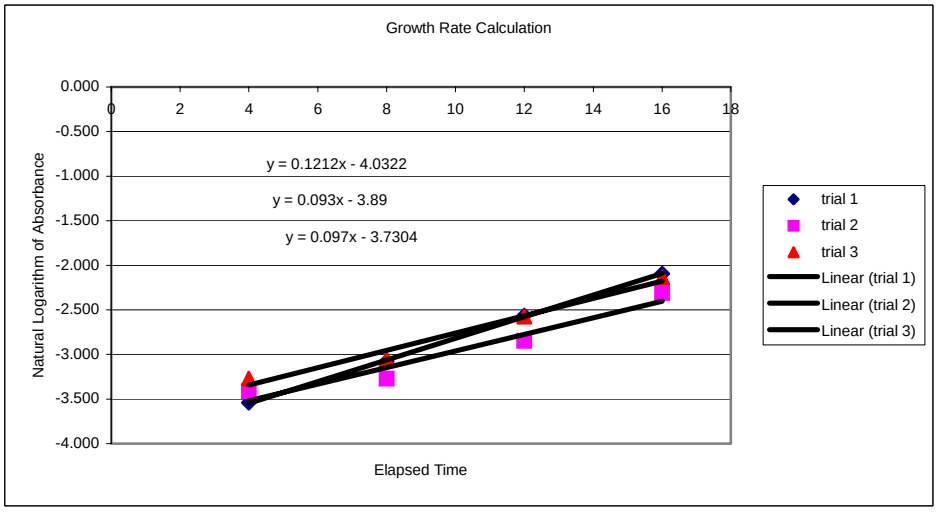


trial 1	trial 2	trial 3	Growth Rate	STD
0.4735	0.4584	0.4596	0.4638	0.0084

70 g (0.1)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.026	0.032	0.035	-3.650	-3.442	-3.352
4	0.029	0.033	0.038	-3.540	-3.411	-3.270
8	0.046	0.038	0.047	-3.079	-3.270	-3.058
12	0.077	0.058	0.076	-2.564	-2.847	-2.577
16	0.123	0.099	0.118	-2.096	-2.313	-2.137
20	0.154	0.124	0.123	-1.871	-2.087	-2.096
24	0.160	0.134	0.134	-1.833	-2.010	-2.010
28	0.171	0.128	0.160	-1.766	-2.056	-1.833

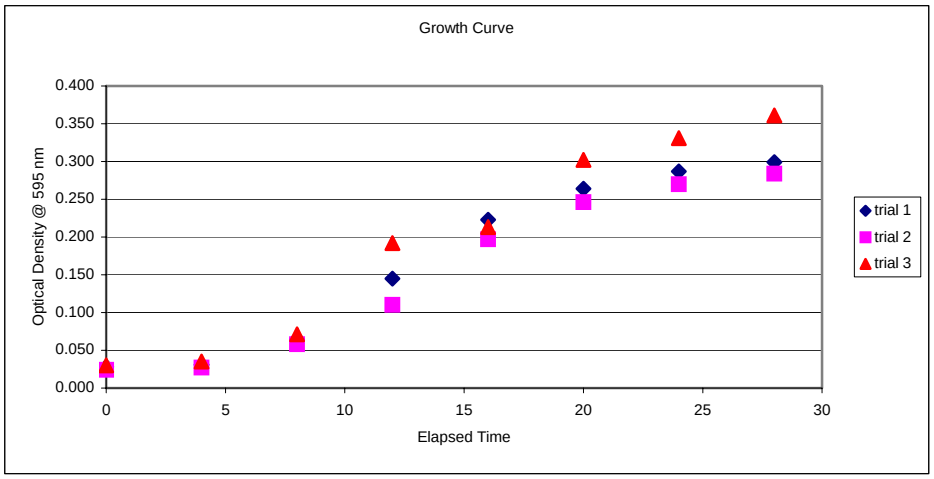


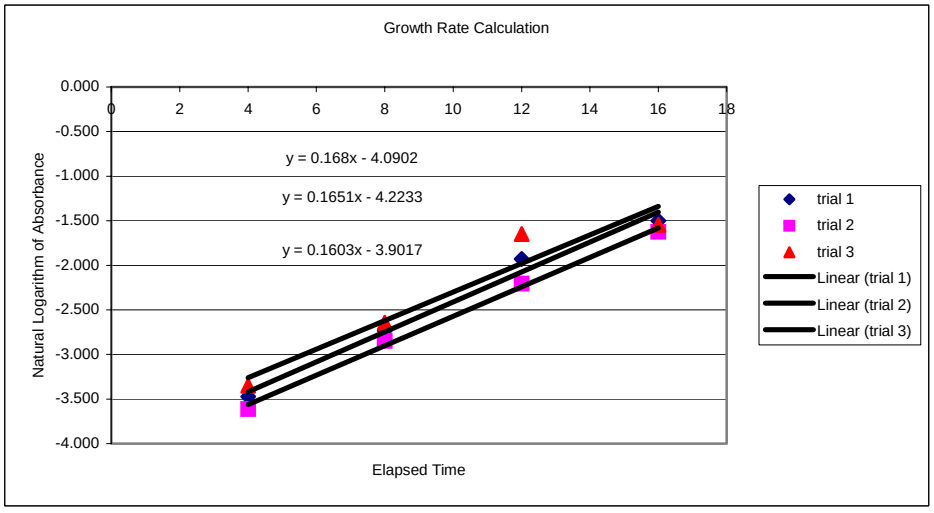


trial 1	trial 2	trial 3	Growth Rate	STD
0.1212	0.0930	0.0970	0.1037	0.0153

70 g (0.25)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.028	0.024	0.030	-3.576	-3.730	-3.507
4	0.031	0.027	0.035	-3.474	-3.612	-3.352
8	0.065	0.058	0.071	-2.733	-2.847	-2.645
12	0.145	0.110	0.192	-1.931	-2.207	-1.650
16	0.223	0.197	0.213	-1.501	-1.625	-1.546
20	0.264	0.246	0.302	-1.332	-1.402	-1.197
24	0.287	0.270	0.331	-1.248	-1.309	-1.106
28	0.299	0.284	0.361	-1.207	-1.259	-1.019

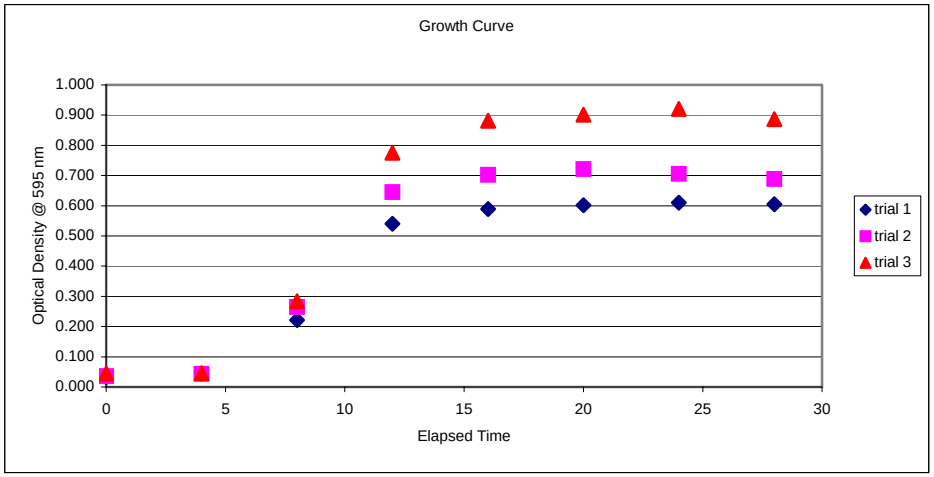


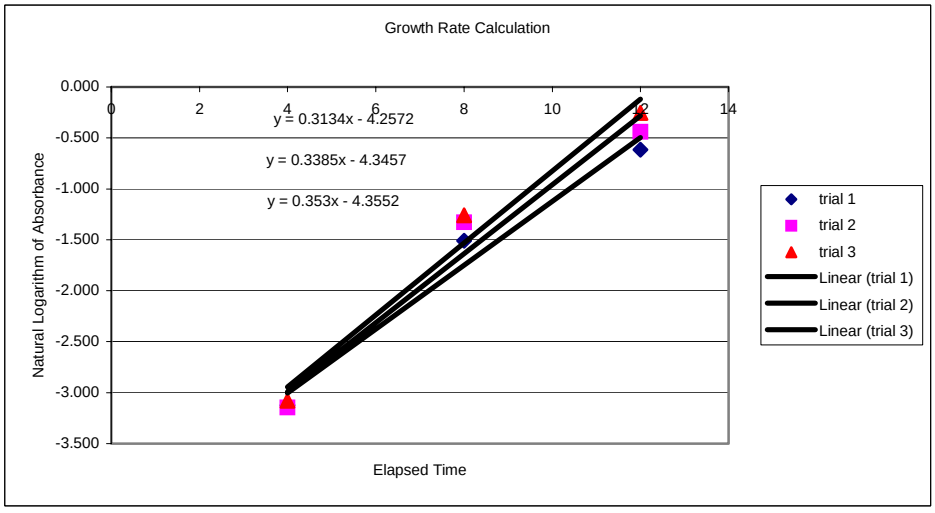


trial 1	trial 2	trial 3	Growth Rate	STD
0.1680	0.1668	0.1603	0.1650	0.0041

70 g (1)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.033	0.036	0.044	-3.411	-3.324	-3.124
4	0.044	0.043	0.046	-3.124	-3.147	-3.079
8	0.221	0.265	0.284	-1.510	-1.328	-1.259
12	0.540	0.645	0.775	-0.616	-0.439	-0.255
16	0.589	0.702	0.881	-0.529	-0.354	-0.127
20	0.602	0.721	0.902	-0.507	-0.327	-0.103
24	0.610	0.705	0.921	-0.494	-0.350	-0.082
28	0.605	0.689	0.887	-0.503	-0.373	-0.120

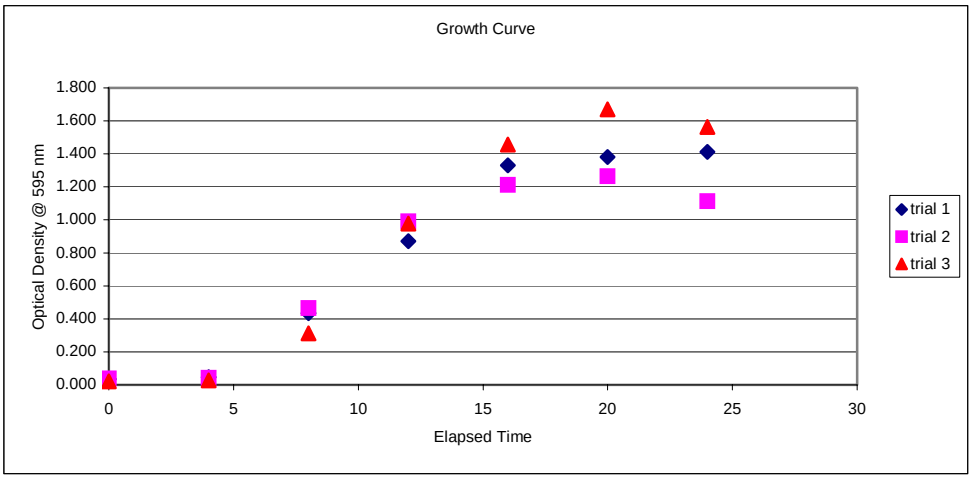


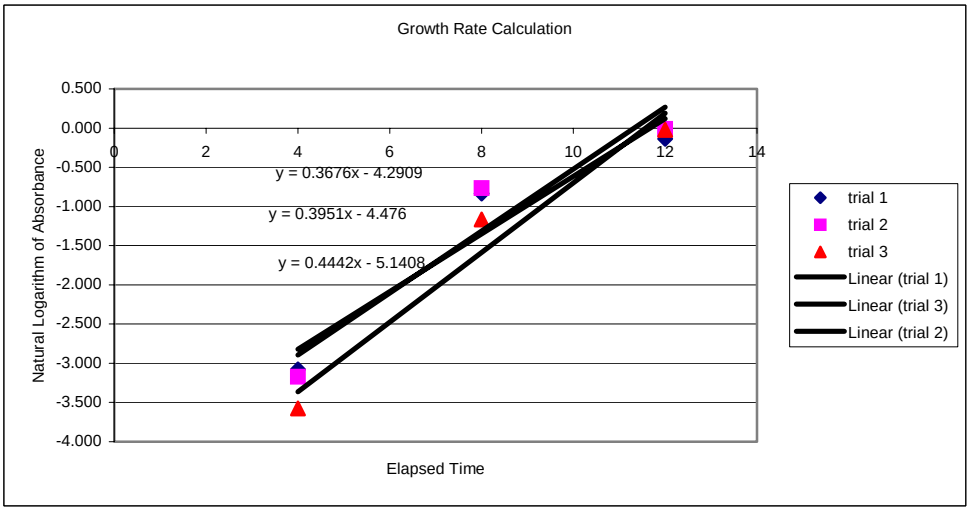


trial 1	trial 2	trial 3	Growth Rate	STD
0.3134	0.3385	0.3530	0.3350	0.0200

70 g (2)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.035	0.038	0.022	-3.352	-3.270	-3.817
4	0.046	0.042	0.028	-3.079	-3.170	-3.576
8	0.435	0.465	0.312	-0.832	-0.766	-1.165
12	0.871	0.991	0.978	-0.138	-0.009	-0.022
16	1.331	1.213	1.456	0.286	0.193	0.376
20	1.381	1.264	1.670	0.323	0.234	0.513
24	1.412	1.113	1.564	0.345	0.107	0.447

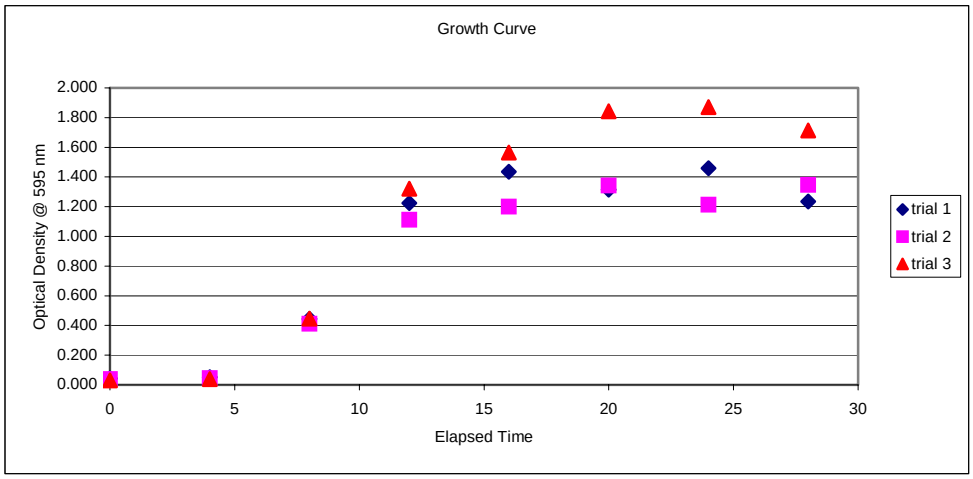


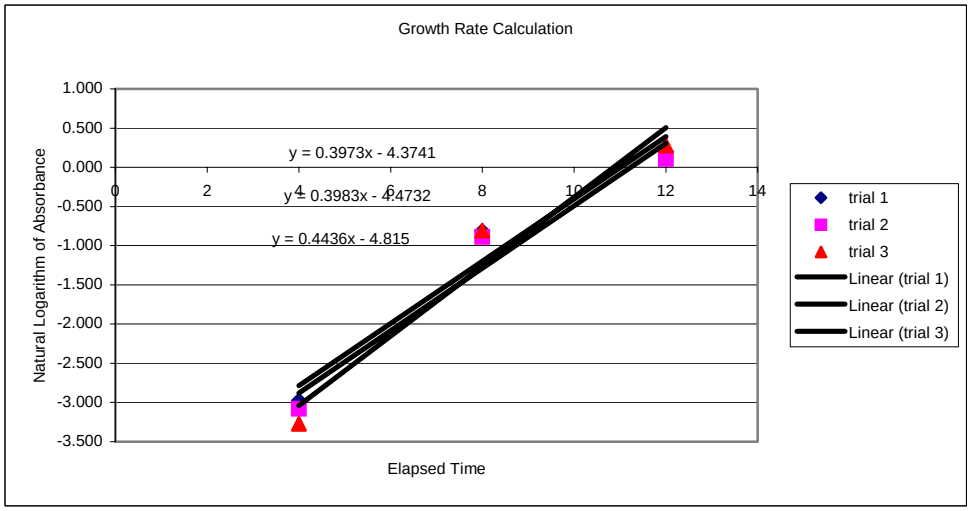


trial 1	trial 2	trial 3	Growth Rate	STD
0.3676	0.3951	0.4442	0.4023	0.0388

70 g (5)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.044	0.039	0.030	-3.124	-3.244	-3.507
4	0.051	0.046	0.038	-2.976	-3.079	-3.270
8	0.443	0.411	0.446	-0.814	-0.889	-0.807
12	1.224	1.113	1.321	0.202	0.107	0.278
16	1.436	1.200	1.564	0.362	0.182	0.447
20	1.315	1.342	1.843	0.274	0.294	0.611
24	1.460	1.214	1.871	0.378	0.194	0.626
28	1.234	1.346	1.713	0.210	0.297	0.538

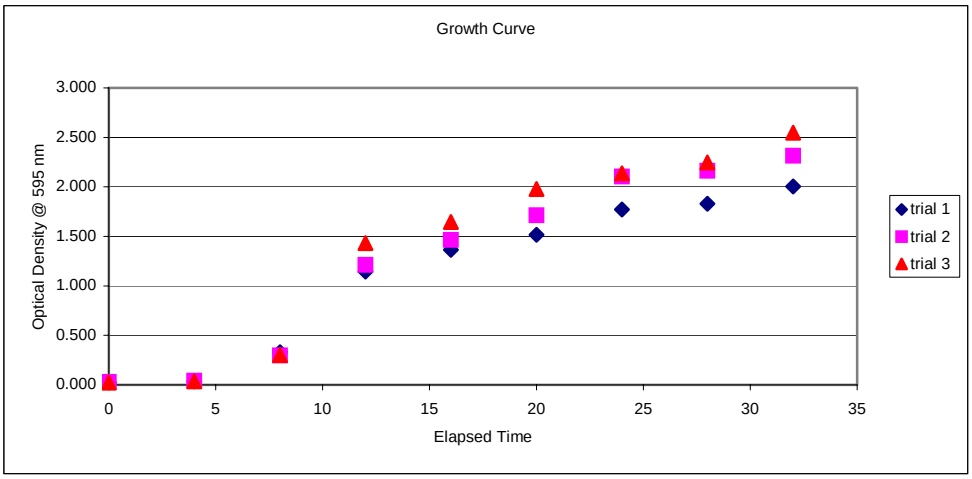


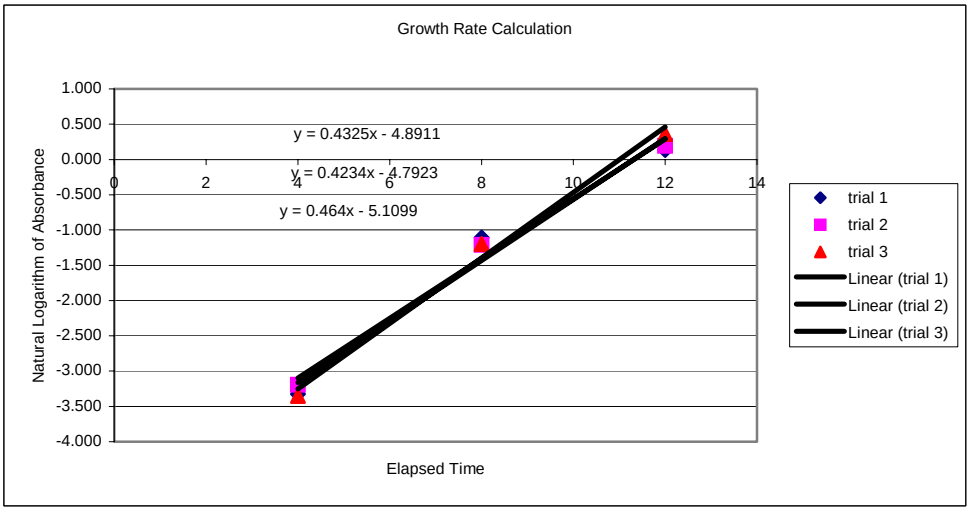


trial 1	trial 2	trial 3	Growth Rate	STD
0.3973	0.3983	0.4436	0.4131	0.0264

70 g (10)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.025	0.029	0.024	-3.689	-3.540	-3.730
4	0.036	0.041	0.035	-3.324	-3.194	-3.352
8	0.331	0.297	0.301	-1.106	-1.214	-1.201
12	1.145	1.213	1.433	0.135	0.193	0.360
16	1.364	1.465	1.645	0.310	0.382	0.498
20	1.516	1.713	1.977	0.416	0.538	0.682
24	1.770	2.105	2.136	0.571	0.744	0.759
28	1.831	2.164	2.246	0.605	0.772	0.809
32	2.003	2.314	2.547	0.695	0.839	0.935

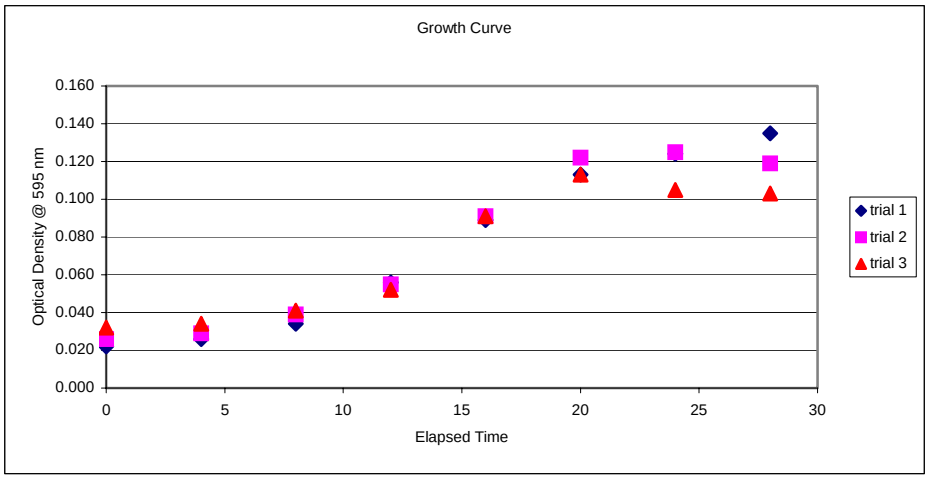


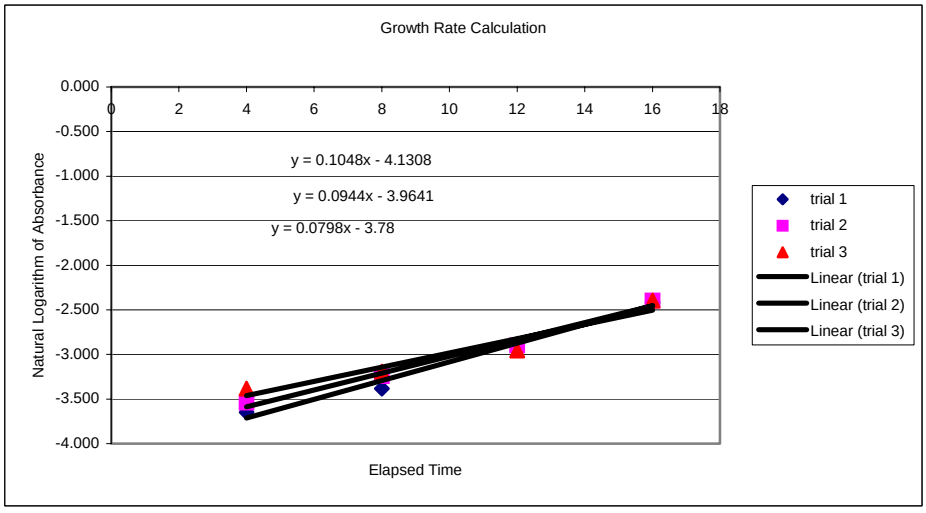


trial 1	trial 2	trial 3	Growth Rate	STD
0.4325	0.4234	0.4640	0.4400	0.0213

90 g (0.1)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.022	0.026	0.032	-3.817	-3.650	-3.442
4	0.026	0.029	0.034	-3.650	-3.540	-3.381
8	0.034	0.039	0.041	-3.381	-3.244	-3.194
12	0.056	0.055	0.052	-2.882	-2.900	-2.957
16	0.089	0.091	0.091	-2.419	-2.397	-2.397
20	0.113	0.122	0.113	-2.180	-2.104	-2.180
24	0.124	0.125	0.105	-2.087	-2.079	-2.254
28	0.135	0.119	0.103	-2.002	-2.129	-2.273

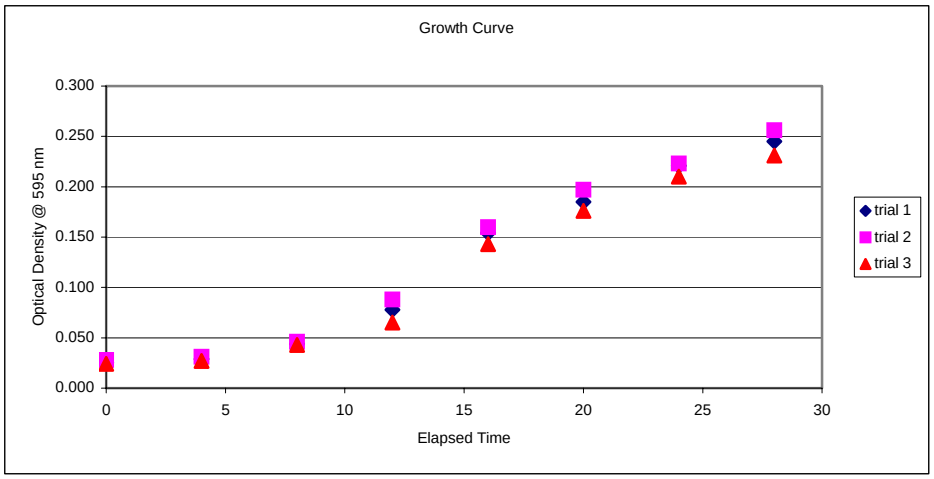


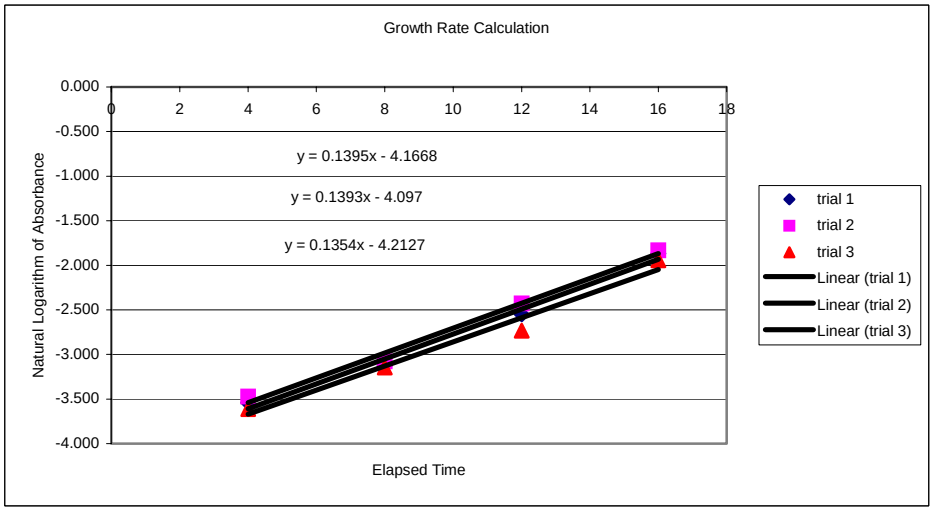


trial 1	trial 2	trial 3	Growth Rate	STD
0.1048	0.0944	0.0798	0.0930	0.0126

90 g (0.25)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.025	0.028	0.024	-3.689	-3.576	-3.730
4	0.029	0.031	0.027	-3.540	-3.474	-3.612
8	0.044	0.046	0.043	-3.124	-3.079	-3.147
12	0.078	0.088	0.065	-2.551	-2.430	-2.733
16	0.154	0.160	0.143	-1.871	-1.833	-1.945
20	0.185	0.197	0.176	-1.687	-1.625	-1.737
24	0.221	0.223	0.210	-1.510	-1.501	-1.561
28	0.245	0.256	0.231	-1.406	-1.363	-1.465

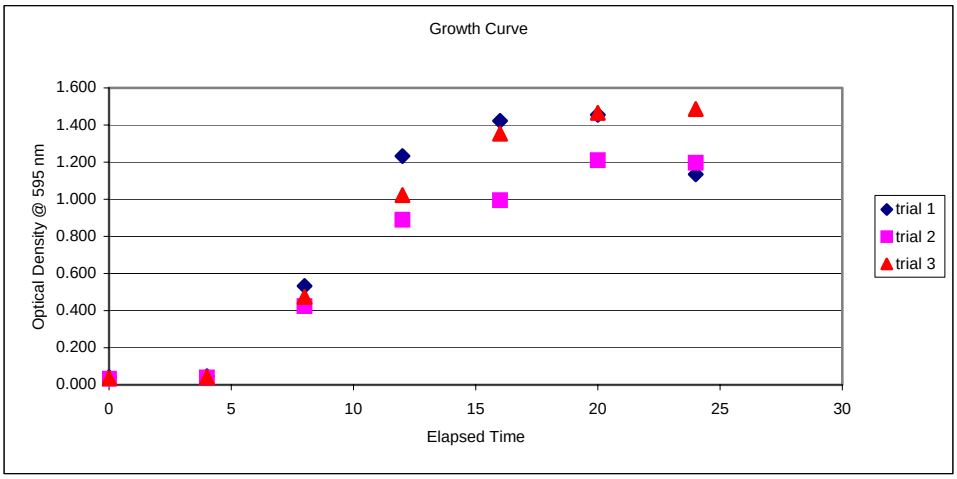


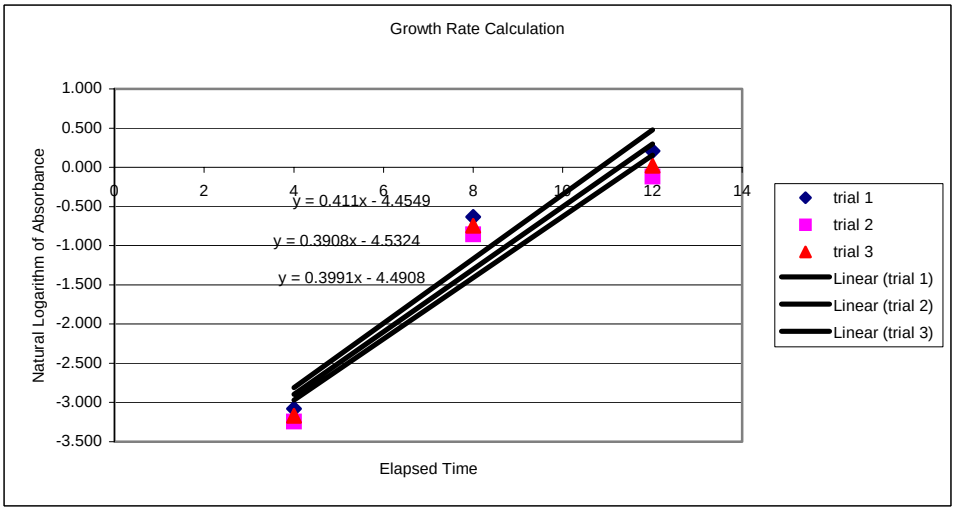


trial 1	trial 2	trial 3	Growth Rate	STD
0.1395	0.1393	0.1354	0.1381	0.0023

90 g (2)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.041	0.033	0.036	-3.194	-3.411	-3.324
4	0.046	0.039	0.042	-3.079	-3.244	-3.170
8	0.532	0.425	0.474	-0.631	-0.856	-0.747
12	1.232	0.889	1.023	0.209	-0.118	0.023
16	1.422	0.994	1.354	0.352	-0.006	0.303
20	1.455	1.210	1.466	0.375	0.191	0.383
24	1.134	1.197	1.487	0.126	0.180	0.397

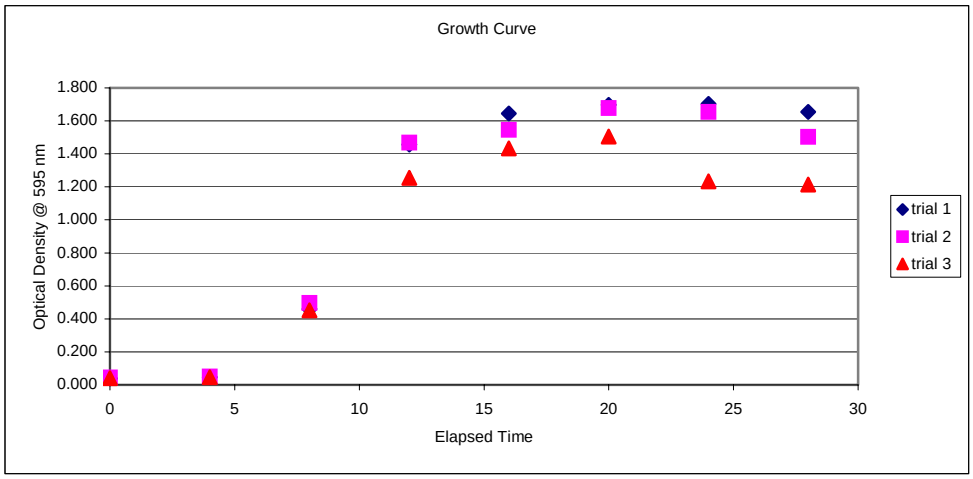


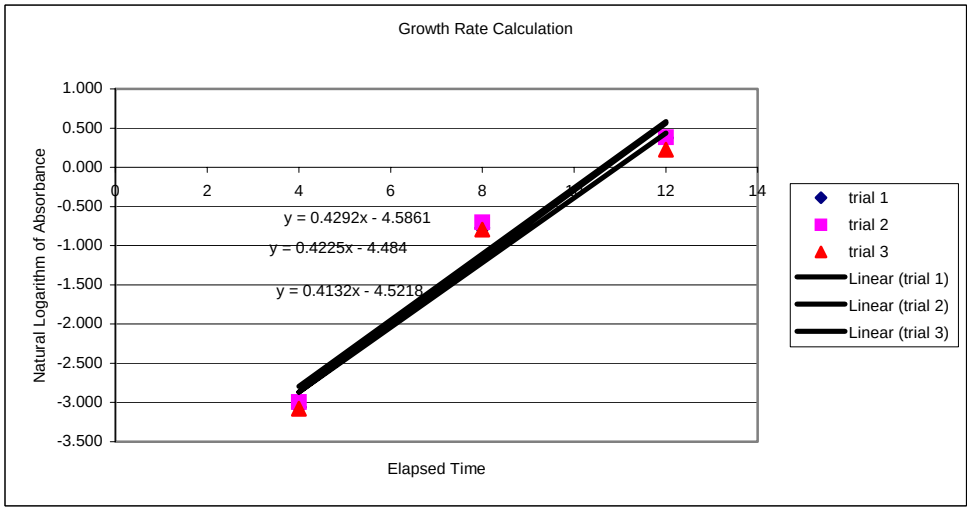


trial 1	trial 2	trial 3	Growth Rate	STD
0.4110	0.3908	0.3991	0.4003	0.0102

90 g (5)

elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.038	0.045	0.040	-3.270	-3.101	-3.219
4	0.047	0.050	0.046	-3.058	-2.996	-3.079
8	0.460	0.496	0.451	-0.777	-0.701	-0.796
12	1.456	1.468	1.254	0.376	0.384	0.226
16	1.645	1.546	1.433	0.498	0.436	0.360
20	1.697	1.677	1.505	0.529	0.517	0.409
24	1.703	1.654	1.233	0.532	0.503	0.209
28	1.654	1.503	1.214	0.503	0.407	0.194

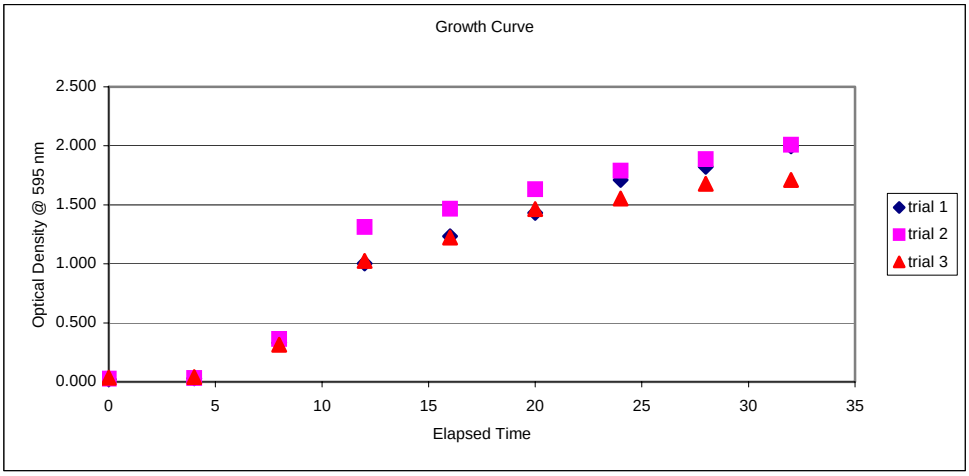


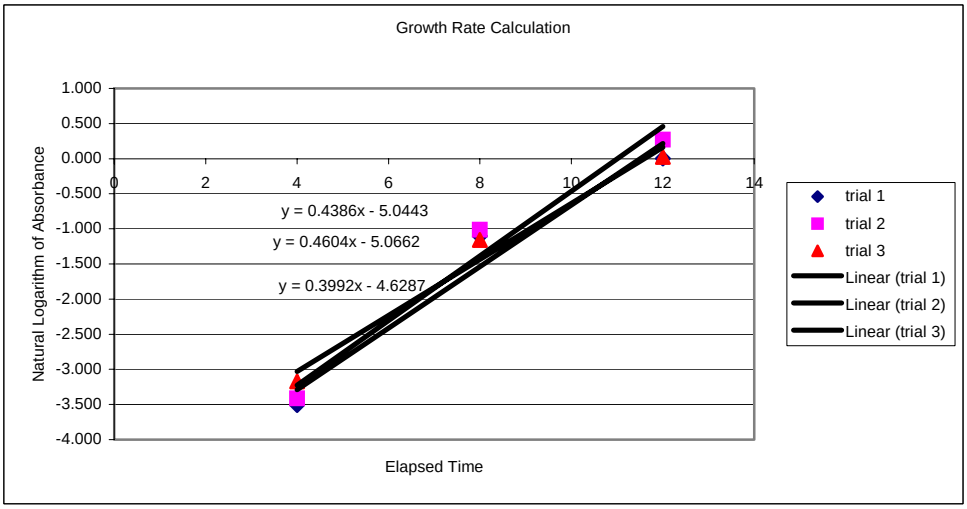


trial 1	trial 2	trial 3	Growth Rate	STD
0.4292	0.4225	0.4132	0.4216	0.0080

90 g (10)

	Absorbance			Natural Logarithms		
Elapsed Time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.023	0.026	0.035	-3.772	-3.650	-3.352
4	0.030	0.033	0.042	-3.507	-3.411	-3.170
8	0.332	0.364	0.314	-1.103	-1.011	-1.158
12	1.002	1.312	1.024	0.002	0.272	0.024
16	1.234	1.468	1.224	0.210	0.384	0.202
20	1.431	1.632	1.464	0.358	0.490	0.381
24	1.712	1.789	1.554	0.538	0.582	0.441
28	1.821	1.887	1.678	0.599	0.635	0.518
32	1.997	2.009	1.710	0.692	0.698	0.536



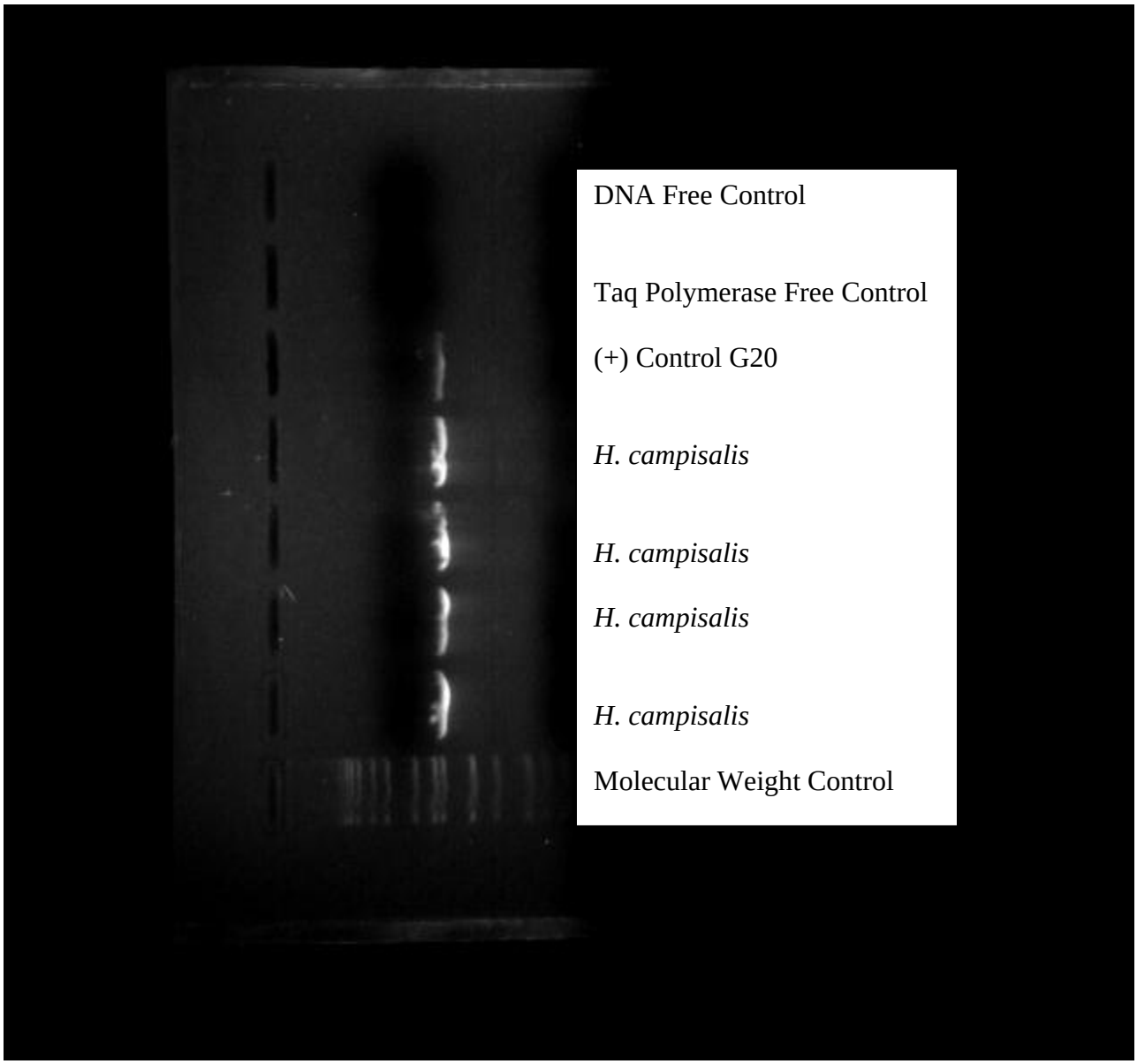


trial 1	trial 2	trial 3	Growth Rate	STD
0.4386	0.4604	0.3992	0.4327	0.0310

Appendix P

In order to verify that pure *H. campisalis* cultures were being used for analysis. 16S rRNA sequencing was performed to identify the samples. DNA was extracted from bacteria cultures using a genomic DNA extraction kit. This DNA was subjected to a polymerase chain reaction (PCR) with the results being sequenced and ran through BLAST software.

16S RNA PCR amplification of *H. campisalis*



16S rRNA Sequence

TCGCNGCCTACCATGCAGTCGAGCGGAACGATGGNAGCTTGCTTCCAGGCGT
CGAGCGGCGGACGGGTGAGTAATGCAT
AGGAATCTGCCCGGTAGTGGGGGATAACCTGGGGAAACCCAGGCTAATACCG
CATACGTCCTACGGGAGAAAGCAGGGGA
TCTTCGGACCTTGCGCTATCGGATGAGCCTATGTCGGATTAGCTAGTTGGTGA
GGTAATGGCTCACCAAGGCGACGATCC
GTAGCTGGTCTGAGAGGATGATCAGCCACATCGGGACTGAGACACGGCCCGA
ACTCCTACGGGAGGCAGCAGTGGGGAAT
ATTGGACAATGGGGGCAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGG
CTTTCGGGTTGTAAAGCACTTTCAGTGG
GGAAGAAAGCCTTGGGGCTAATACCCTCGAGGAAGGACATCACCCACAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGC
CGCGGTAATACGGAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCG
CGCGTAGGTGGCTTGATAAGCCgGTTGT
GAAAGCCCCGGGCTCAACCTGGGAACGGCATCCGGAACTGTCAGGCTAGAGT
GCAGGAGAGGAAGGTAGAATTCCCGGTG
TAgCGTGAAATGCGTAgAgATCGGGAGGAATACCAGTGGCGAANGCGGCNTT
TTGACTGACNCTGANCTGAGTGCNAAAN
CGTGGTANCAAAAGGATTAAATACCCTGGANTCCCCNCNNAACNATTTCACT
AACNNTGGGCC

Blast Results

Halomonas campisalis 16S ribosomal RNA

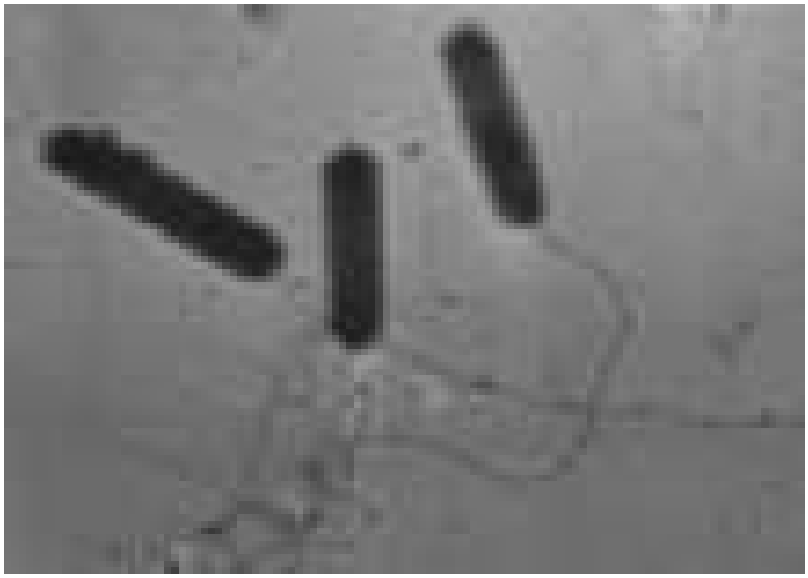
Top of Form

Score = 1269 bits (640), Expect = 0.0
Identities = 666/674 (98%), Gaps = 2/674 (0%)
Strand=Plus/Plus

Appendix Q

This appendix presents physical characteristics of *H. salinarum*. Because growth kinetics were determined using optical density, a correlation relating optical density at 595 nm with dry cell weight is also included.

Characteristics of *H. salinarum* NRC-1



Archaea

Extreme Halophile

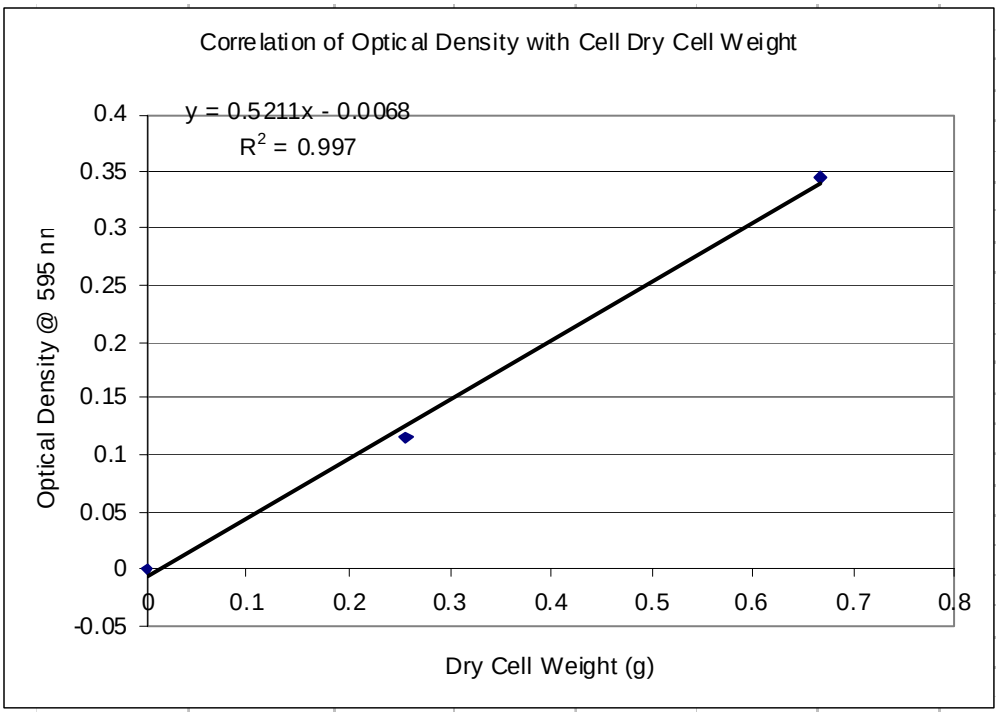
Forms shiny light red circular colonies

Motile Rod

2.5-3.5 μm x 0.5 μm in dimension

G+C content ~ 54.3%

Cell Wt. g/L	Absorbance
0	0
0.257	0.116
0.667	0.345



Appendix R

Appendix R presents the growth medium used to cultivate *H. salinarum*. Substrate (Glucose and sodium nitrate) were not included in this table as they varied from aerobic to denitrifying conditions.

Growth medium for *H. salinarum* NRC-1

For 1 Liter

Sodium Chloride	0-250.0 g/L
Magnesium Sulfate	20.0 g/L
Trisodium citrate	3.0 g/L
Potassium Cloride	2.0 g/L
Tryptone	5.0 g/L
Yeast extract	3.0 g/L

Add Distilled Water to 1 Liter

Autoclave at 121 degrees Celsius for 15 minutes.
Allow to cool to room temperature

Add 0.1 ml of Trace Metals (See below) to sterile basal medium.

Trace Metals:

Zinc Sulfate	1.32 g/L
Manganese Sulfate	0.34 g/L
Fe(NO4)(SO4)2 . 6H2O	0.78 g/L
Cupric Sulfate	0.14 g/L
Distilled water	200.0 ml

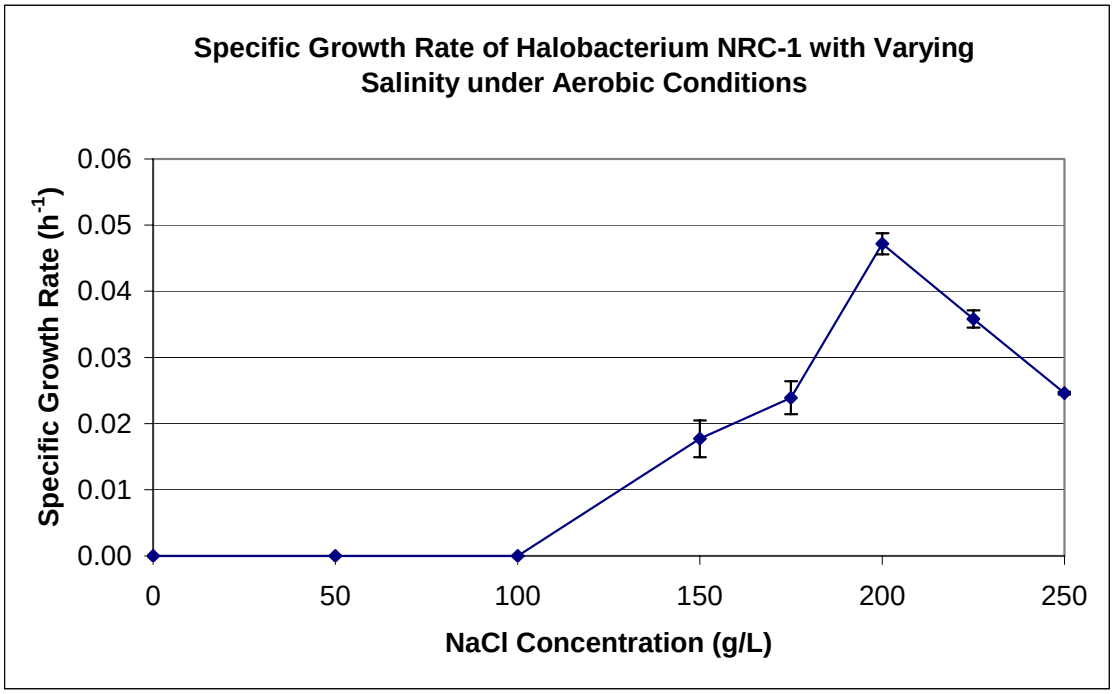
pH adjusted to 7.0 using sodium hydroxide and/or hydrochloric acid

Appendix S

Appendix S presents optical density data gathered for the growth of *H. salinarum* over a wide range of salinities in the growth medium under aerobic conditions. All experiments were performed at pH 7, as this was the pH at which growth optima was earlier reported to occur at. The maximum specific growth rate was determined by plotting the natural logarithms of the optical density data versus elapsed growth time. A linear line was then fit to the steepest part of this data set. The slope of this line represented the maximum specific growth rate.

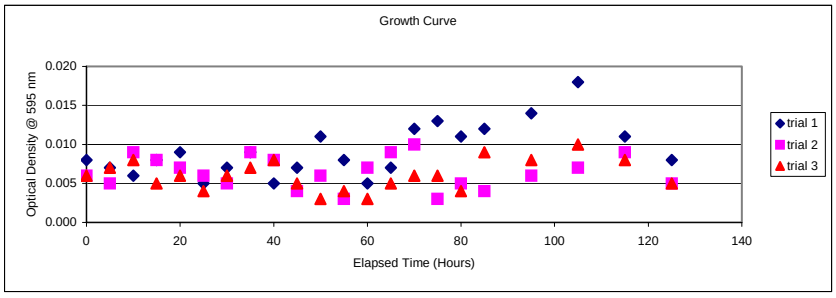
Growth kinetics of *H. salinerum* NRC-1 under varying salinity (aerobic conditions)

NaCl Concentration (g/L)	Growth Rate	STD
0	0.0000	0.0000
50	0.0000	0.0000
100	0.0000	0.0000
150	0.0177	0.0028
175	0.0239	0.0025
200	0.0472	0.0016
225	0.0358	0.0013
250	0.0246	0.0002



0 g/L

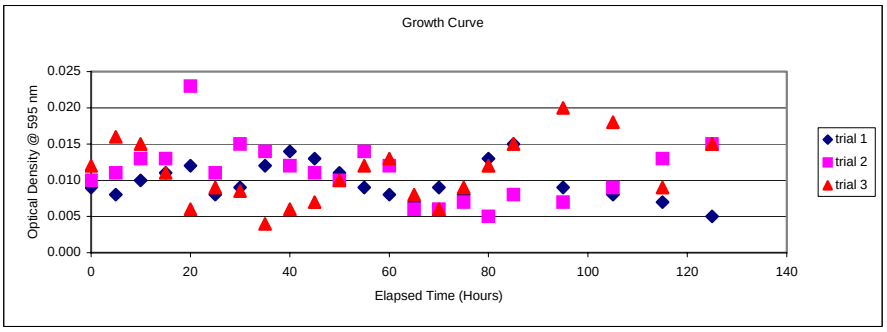
				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.008	0.006	0.006	-4.828	-5.116	-5.116
5	0.007	0.005	0.007	-4.962	-5.298	-4.962
10	0.006	0.009	0.008	-5.116	-4.711	-4.828
15	0.008	0.008	0.005	-4.828	-4.828	-5.298
20	0.009	0.007	0.006	-4.711	-4.962	-5.116
25	0.005	0.006	0.004	-5.298	-5.116	-5.521
30	0.007	0.005	0.006	-4.962	-5.298	-5.116
35	0.009	0.009	0.007	-4.711	-4.711	-4.962
40	0.005	0.008	0.008	-5.298	-4.828	-4.828
45	0.007	0.004	0.005	-4.962	-5.521	-5.298
50	0.011	0.006	0.003	-4.510	-5.116	-5.809
55	0.008	0.003	0.004	-4.828	-5.809	-5.521
60	0.005	0.007	0.003	-5.298	-4.962	-5.809
65	0.007	0.009	0.005	-4.962	-4.711	-5.298
70	0.012	0.010	0.006	-4.423	-4.605	-5.116
75	0.013	0.003	0.006	-4.343	-5.809	-5.116
80	0.011	0.005	0.004	-4.510	-5.298	-5.521
85	0.012	0.004	0.009	-4.423	-5.521	-4.711
95	0.014	0.006	0.008	-4.269	-5.116	-4.828
105	0.018	0.007	0.010	-4.017	-4.962	-4.605
115	0.011	0.009	0.008	-4.510	-4.711	-4.828
125	0.008	0.005	0.005	-4.828	-5.298	-5.298



	Growth Rate
trial 1	0
trial 2	0
trial 3	0
Average	0.0000
STD	0.0000

50 g/L

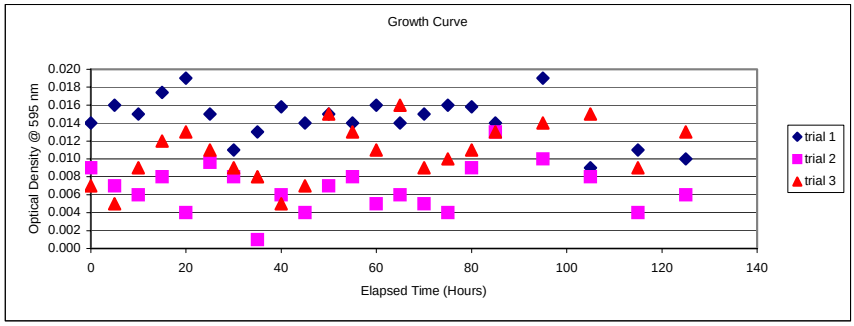
				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.009	0.010	0.012	-4.711	-4.605	-4.423
5	0.008	0.011	0.016	-4.828	-4.510	-4.135
10	0.010	0.013	0.015	-4.605	-4.343	-4.200
15	0.011	0.013	0.011	-4.510	-4.343	-4.510
20	0.012	0.023	0.006	-4.423	-3.772	-5.116
25	0.008	0.011	0.009	-4.828	-4.510	-4.711
30	0.009	0.015	0.009	-4.711	-4.200	-4.768
35	0.012	0.014	0.004	-4.423	-4.269	-5.521
40	0.014	0.012	0.006	-4.269	-4.423	-5.116
45	0.013	0.011	0.007	-4.343	-4.510	-4.962
50	0.011	0.010	0.010	-4.510	-4.605	-4.605
55	0.009	0.014	0.012	-4.711	-4.269	-4.423
60	0.008	0.012	0.013	-4.828	-4.423	-4.343
65	0.007	0.006	0.008	-4.962	-5.116	-4.828
70	0.009	0.006	0.006	-4.711	-5.116	-5.116
75	0.008	0.007	0.009	-4.828	-4.962	-4.711
80	0.013	0.005	0.012	-4.343	-5.298	-4.423
85	0.015	0.008	0.015	-4.200	-4.828	-4.200
95	0.009	0.007	0.020	-4.711	-4.962	-3.912
105	0.008	0.009	0.018	-4.828	-4.711	-4.017
115	0.007	0.013	0.009	-4.962	-4.343	-4.711
125	0.005	0.015	0.015	-5.298	-4.200	-4.200



	Growth Rate
trial 1	0
trial 2	0
trial 3	0
Average	0.0000
STD	0.0000

100 g/L

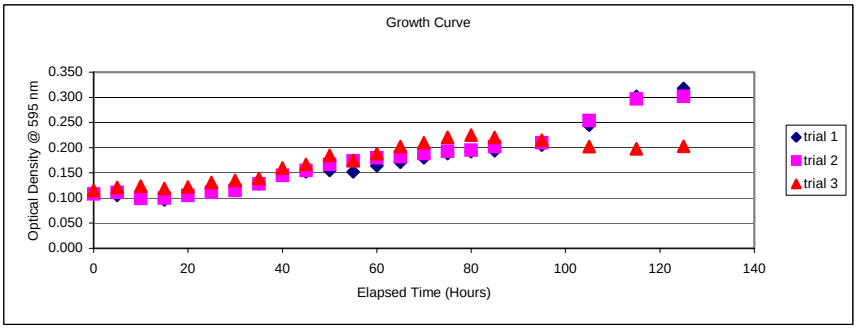
				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.014	0.009	0.007	-4.269	-4.711	-4.962
5	0.016	0.007	0.005	-4.135	-4.962	-5.298
10	0.015	0.006	0.009	-4.200	-5.116	-4.711
15	0.017	0.008	0.012	-4.051	-4.828	-4.423
20	0.019	0.004	0.013	-3.963	-5.521	-4.343
25	0.015	0.010	0.011	-4.200	-4.646	-4.510
30	0.011	0.008	0.009	-4.510	-4.828	-4.711
35	0.013	0.001	0.008	-4.343	-6.908	-4.828
40	0.016	0.006	0.005	-4.148	-5.116	-5.298
45	0.014	0.004	0.007	-4.269	-5.521	-4.962
50	0.015	0.007	0.015	-4.200	-4.962	-4.200
55	0.014	0.008	0.013	-4.269	-4.828	-4.343
60	0.016	0.005	0.011	-4.135	-5.298	-4.510
65	0.014	0.006	0.016	-4.269	-5.116	-4.135
70	0.015	0.005	0.009	-4.200	-5.298	-4.711
75	0.016	0.004	0.010	-4.135	-5.521	-4.605
80	0.016	0.009	0.011	-4.148	-4.711	-4.510
85	0.014	0.013	0.013	-4.269	-4.343	-4.343
95	0.019	0.010	0.014	-3.963	-4.605	-4.269
105	0.009	0.008	0.015	-4.711	-4.828	-4.200
115	0.011	0.004	0.009	-4.510	-5.521	-4.711
125	0.010	0.006	0.013	-4.605	-5.116	-4.343



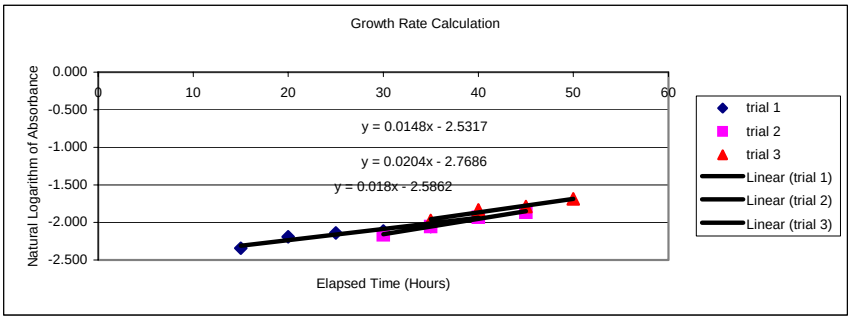
	Growth Rate
trial 1	0
trial 2	0
trial 3	0
Average	0.0000
STD	0.0000

150 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.112	0.108	0.115	-2.189	-2.226	-2.163
5	0.105	0.111	0.121	-2.254	-2.198	-2.112
10	0.111	0.099	0.124	-2.198	-2.313	-2.087
15	0.096	0.100	0.119	-2.343	-2.303	-2.129
20	0.112	0.105	0.122	-2.189	-2.254	-2.104
25	0.118	0.111	0.131	-2.137	-2.198	-2.033
30	0.121	0.115	0.135	-2.112	-2.163	-2.002
35	0.128	0.128	0.139	-2.056	-2.056	-1.973
40	0.148	0.145	0.160	-1.911	-1.931	-1.833
45	0.152	0.155	0.167	-1.884	-1.864	-1.790
50	0.155	0.167	0.185	-1.864	-1.790	-1.687
55	0.152	0.174	0.175	-1.884	-1.749	-1.743
60	0.164	0.180	0.188	-1.808	-1.715	-1.671
65	0.171	0.182	0.202	-1.766	-1.704	-1.599
70	0.180	0.188	0.210	-1.715	-1.671	-1.561
75	0.188	0.193	0.221	-1.671	-1.645	-1.510
80	0.192	0.195	0.225	-1.650	-1.635	-1.492
85	0.194	0.202	0.220	-1.640	-1.599	-1.514
95	0.205	0.210	0.215	-1.585	-1.561	-1.537
105	0.245	0.254	0.202	-1.406	-1.370	-1.599
115	0.302	0.297	0.198	-1.197	-1.214	-1.619
125	0.318	0.302	0.203	-1.146	-1.197	-1.595

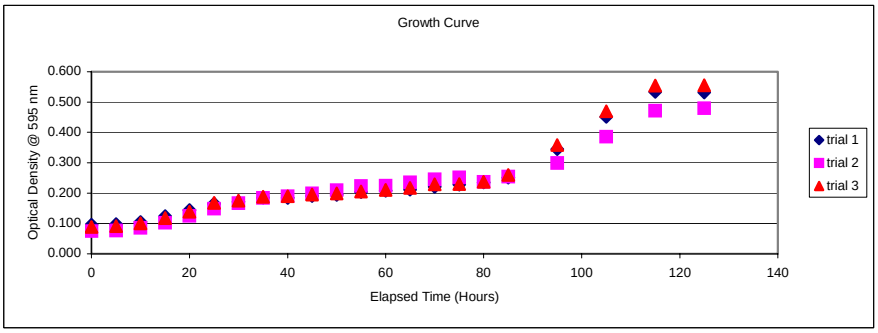


	Growth Rate
trial 1	0.0148
trial 2	0.0204
trial 3	0.018
Average	0.0177
STD	0.0028

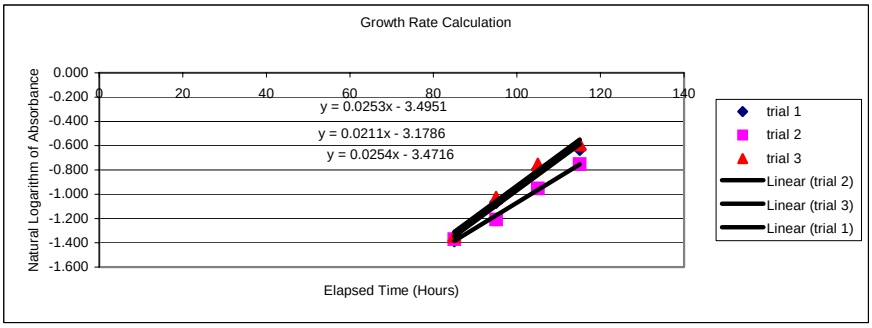


175 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.096	0.075	0.088	-2.343	-2.590	-2.430
5	0.098	0.077	0.091	-2.323	-2.564	-2.397
10	0.105	0.086	0.100	-2.254	-2.453	-2.303
15	0.124	0.102	0.118	-2.087	-2.283	-2.137
20	0.144	0.125	0.139	-1.938	-2.079	-1.973
25	0.165	0.149	0.168	-1.802	-1.904	-1.784
30	0.171	0.167	0.175	-1.766	-1.790	-1.743
35	0.185	0.184	0.188	-1.687	-1.693	-1.671
40	0.184	0.190	0.190	-1.693	-1.661	-1.661
45	0.191	0.199	0.195	-1.655	-1.614	-1.635
50	0.194	0.210	0.199	-1.640	-1.561	-1.614
55	0.203	0.223	0.205	-1.595	-1.501	-1.585
60	0.208	0.224	0.211	-1.570	-1.496	-1.556
65	0.212	0.235	0.217	-1.551	-1.446	-1.528
70	0.221	0.245	0.229	-1.510	-1.406	-1.474
75	0.228	0.252	0.230	-1.478	-1.378	-1.470
80	0.235	0.237	0.237	-1.448	-1.440	-1.440
85	0.251	0.254	0.260	-1.382	-1.370	-1.347
95	0.345	0.299	0.358	-1.064	-1.207	-1.027
105	0.451	0.386	0.470	-0.796	-0.952	-0.755
115	0.533	0.471	0.554	-0.629	-0.753	-0.591
125	0.532	0.480	0.555	-0.631	-0.734	-0.589

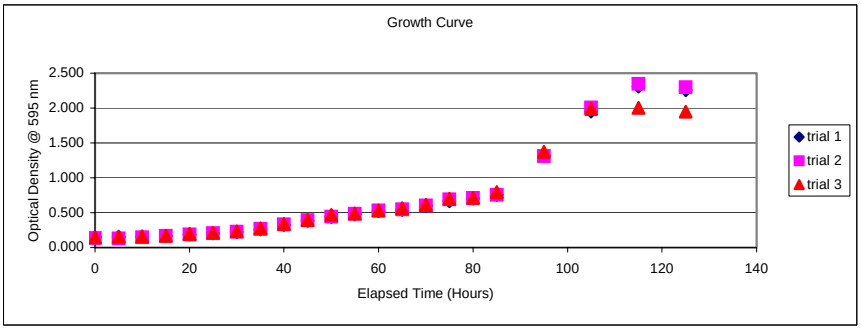


	Growth Rate
trial 1	0.0253
trial 2	0.0211
trial 3	0.0254
Average	0.0239
STD	0.0025

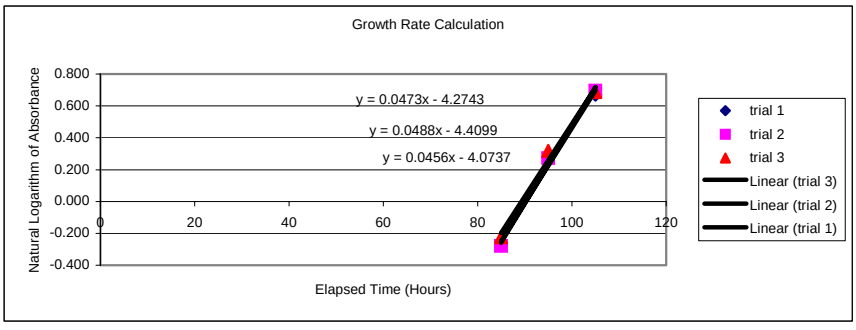


200 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.148	0.135	0.148	-1.911	-2.002	-1.911
5	0.151	0.133	0.155	-1.890	-2.017	-1.864
10	0.155	0.149	0.160	-1.864	-1.904	-1.833
15	0.164	0.167	0.168	-1.808	-1.790	-1.784
20	0.189	0.188	0.195	-1.666	-1.671	-1.635
25	0.203	0.210	0.211	-1.595	-1.561	-1.556
30	0.225	0.229	0.234	-1.492	-1.474	-1.452
35	0.262	0.270	0.278	-1.339	-1.309	-1.280
40	0.324	0.333	0.339	-1.127	-1.100	-1.082
45	0.384	0.391	0.398	-0.957	-0.939	-0.921
50	0.441	0.445	0.467	-0.819	-0.810	-0.761
55	0.477	0.485	0.488	-0.740	-0.724	-0.717
60	0.524	0.534	0.534	-0.646	-0.627	-0.627
65	0.542	0.550	0.564	-0.612	-0.598	-0.573
70	0.598	0.605	0.611	-0.514	-0.503	-0.493
75	0.665	0.691	0.700	-0.408	-0.370	-0.357
80	0.705	0.711	0.712	-0.350	-0.341	-0.340
85	0.758	0.756	0.798	-0.277	-0.280	-0.226
95	1.321	1.314	1.374	0.278	0.273	0.318
105	1.954	2.008	1.987	0.670	0.697	0.687
115	2.310	2.347	2.005	0.837	0.853	0.696
125	2.258	2.301	1.947	0.814	0.833	0.666

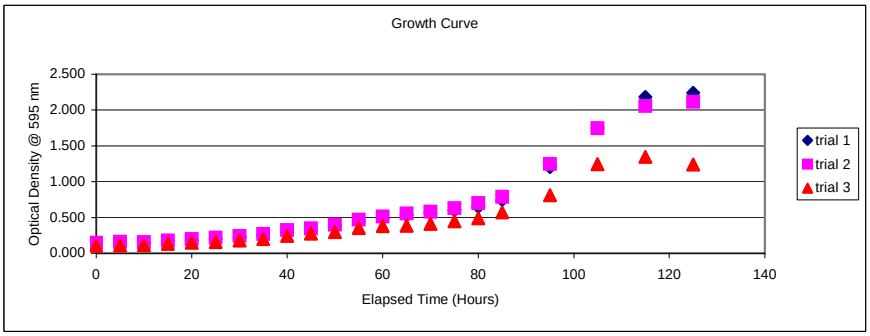


	Growth Rate
trial 1	0.0473
trial 2	0.0488
trial 3	0.0456
Average	0.0472
STD	0.0016

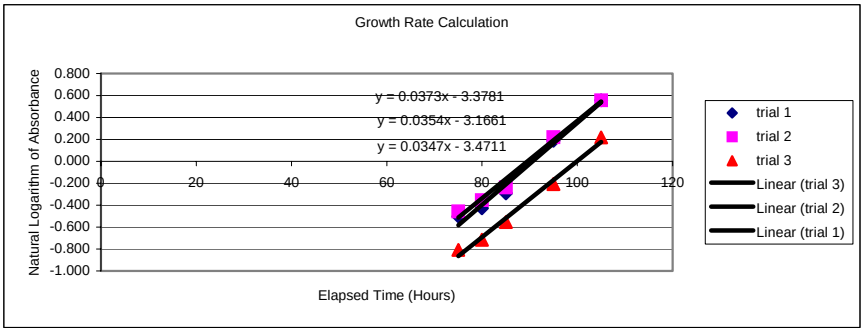


225 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.140	0.151	0.105	-1.966	-1.890	-2.254
5	0.141	0.160	0.111	-1.959	-1.833	-2.198
10	0.145	0.158	0.114	-1.931	-1.845	-2.172
15	0.176	0.180	0.134	-1.737	-1.715	-2.010
20	0.185	0.199	0.148	-1.687	-1.614	-1.911
25	0.202	0.220	0.158	-1.599	-1.514	-1.845
30	0.228	0.245	0.179	-1.478	-1.406	-1.720
35	0.255	0.271	0.201	-1.366	-1.306	-1.604
40	0.300	0.325	0.244	-1.204	-1.124	-1.411
45	0.334	0.351	0.276	-1.097	-1.047	-1.287
50	0.387	0.402	0.297	-0.949	-0.911	-1.214
55	0.455	0.470	0.355	-0.787	-0.755	-1.036
60	0.492	0.515	0.379	-0.709	-0.664	-0.970
65	0.533	0.557	0.385	-0.629	-0.585	-0.955
70	0.564	0.581	0.412	-0.573	-0.543	-0.887
75	0.605	0.633	0.446	-0.503	-0.457	-0.807
80	0.651	0.702	0.489	-0.429	-0.354	-0.715
85	0.746	0.788	0.574	-0.293	-0.238	-0.555
95	1.206	1.247	0.812	0.187	0.221	-0.208
105	1.746	1.746	1.245	0.557	0.557	0.219
115	2.181	2.057	1.347	0.780	0.721	0.298
125	2.240	2.115	1.241	0.806	0.749	0.216

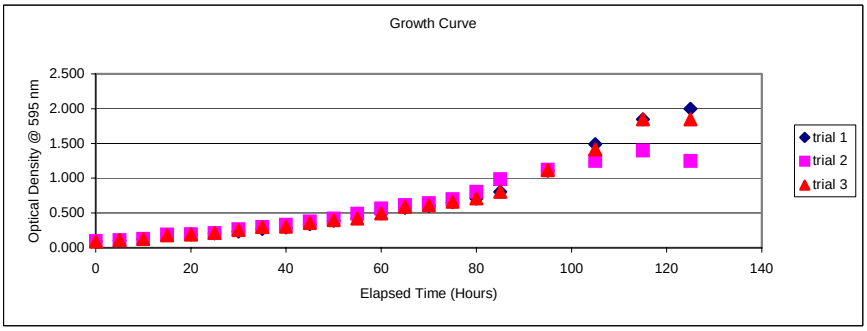


	Growth Rate
trial 1	0.0373
trial 2	0.0354
trial 3	0.0347
Average	0.0358
STD	0.0013

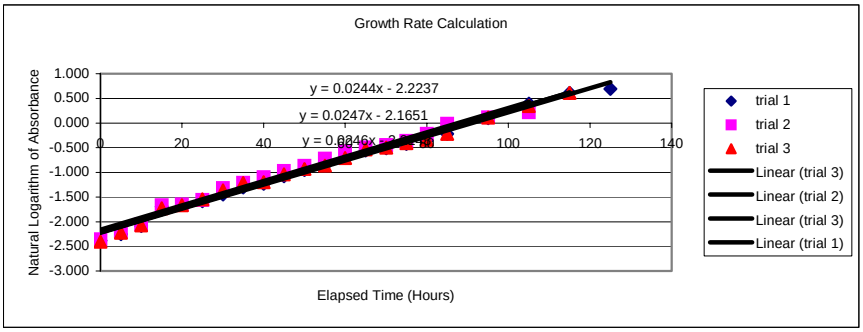


250 g/L

				Logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.092	0.095	0.090	-2.386	-2.354	-2.408
5	0.105	0.111	0.108	-2.254	-2.198	-2.226
10	0.124	0.128	0.125	-2.087	-2.056	-2.079
15	0.184	0.189	0.177	-1.693	-1.666	-1.732
20	0.190	0.192	0.189	-1.661	-1.650	-1.666
25	0.205	0.210	0.213	-1.585	-1.561	-1.546
30	0.234	0.267	0.255	-1.452	-1.321	-1.366
35	0.271	0.298	0.297	-1.306	-1.211	-1.214
40	0.292	0.333	0.302	-1.231	-1.100	-1.197
45	0.340	0.378	0.354	-1.079	-0.973	-1.038
50	0.386	0.420	0.394	-0.952	-0.868	-0.931
55	0.425	0.487	0.421	-0.856	-0.719	-0.865
60	0.492	0.564	0.493	-0.709	-0.573	-0.707
65	0.574	0.612	0.588	-0.555	-0.491	-0.531
70	0.601	0.641	0.608	-0.509	-0.445	-0.498
75	0.654	0.698	0.664	-0.425	-0.360	-0.409
80	0.705	0.805	0.711	-0.350	-0.217	-0.341
85	0.802	0.987	0.803	-0.221	-0.013	-0.219
95	1.108	1.124	1.115	0.103	0.117	0.109
105	1.489	1.249	1.415	0.398	0.222	0.347
115	1.845	1.397	1.846	0.612	0.334	0.613
125	2.000	1.248	1.847	0.693	0.222	0.614



	Growth Rate
trial 1	0.0244
trial 2	0.0247
trial 3	0.0246
Average	0.0246
STD	0.0002

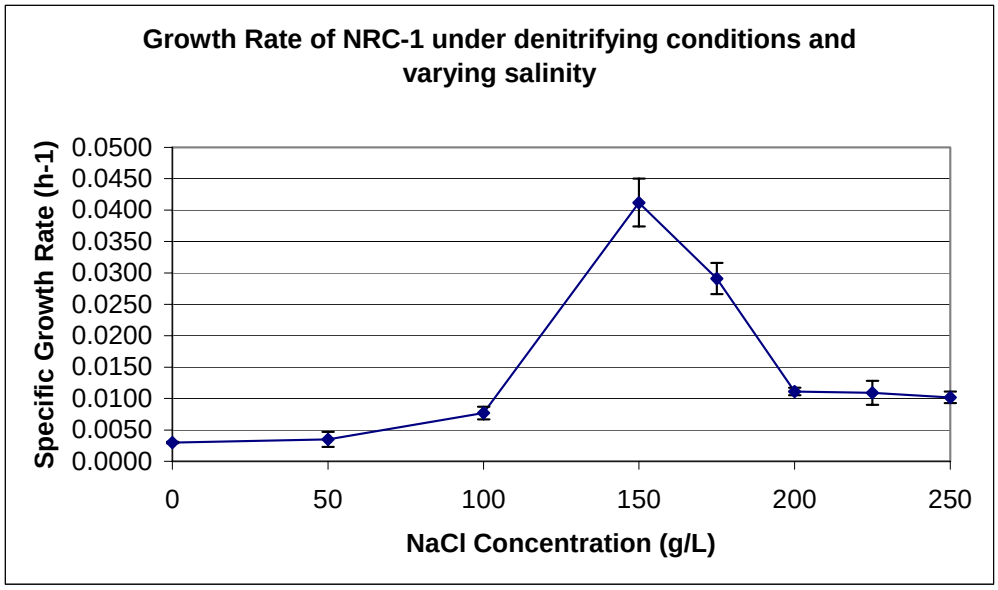


Appendix T

Appendix T presents optical density data gathered for the growth of *H. salinarum* over a wide range of salinities in the growth medium under denitrifying conditions. All experiments were performed at pH 7, as this was the pH at which growth optima was earlier reported to occur at. The maximum specific growth rate was determined by plotting the natural logarithms of the optical density data versus elapsed growth time. A linear line was then fit to the steepest part of this data set. The slope of this line represented the maximum specific growth rate.

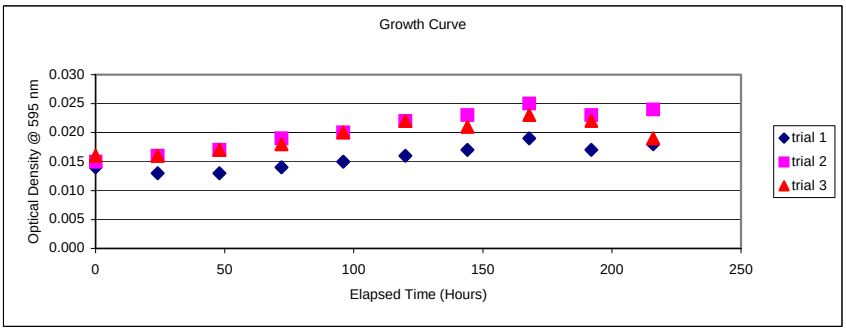
Growth kinetics of *H. salinerium* NRC-1 under varying salinity (denitrifying conditions)

NaCl (g/L)	Growth Rate	STD
0	0.0030	0.0001
50	0.0035	0.0012
100	0.0077	0.0010
150	0.0412	0.0038
175	0.0291	0.0025
200	0.0111	0.0006
225	0.0109	0.0019
250	0.0102	0.0009

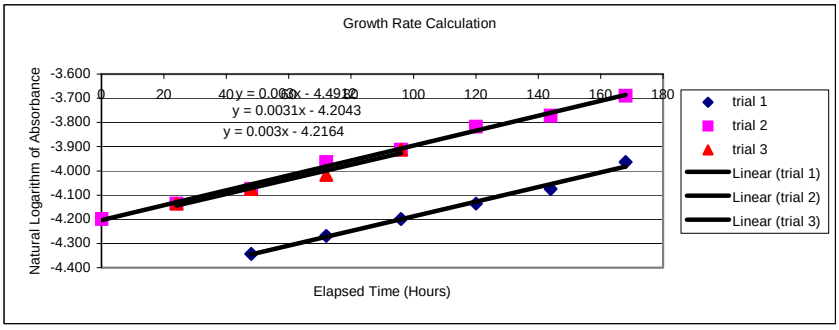


0 g/L

				logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.014	0.015	0.016	-4.269	-4.200	-4.135
24	0.013	0.016	0.016	-4.343	-4.135	-4.135
48	0.013	0.017	0.017	-4.343	-4.075	-4.075
72	0.014	0.019	0.018	-4.269	-3.963	-4.017
96	0.015	0.020	0.020	-4.200	-3.912	-3.912
120	0.016	0.022	0.022	-4.135	-3.817	-3.817
144	0.017	0.023	0.021	-4.075	-3.772	-3.863
168	0.019	0.025	0.023	-3.963	-3.689	-3.772
192	0.017	0.023	0.022	-4.075	-3.772	-3.817
216	0.018	0.024	0.019	-4.017	-3.730	-3.963

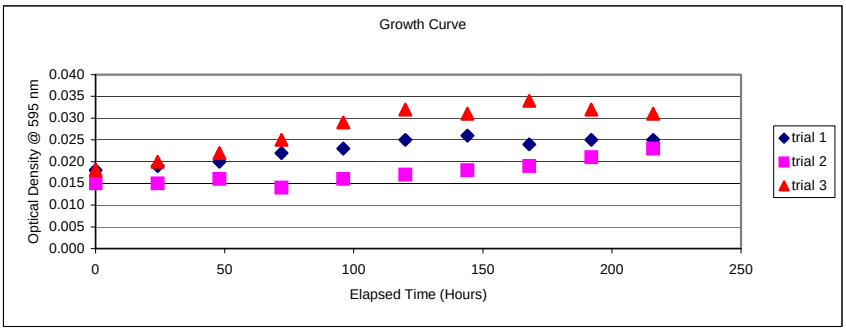


	Growth Rate
trial 1	0.003
trial 2	0.0031
trial 3	0.003
Average	0.0030
STD	0.0001

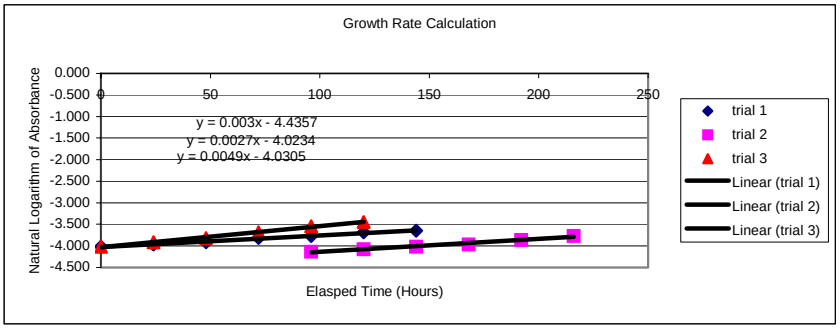


50 g/L

				logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.018	0.015	0.018	-4.017	-4.200	-4.017
24	0.019	0.015	0.020	-3.963	-4.200	-3.912
48	0.020	0.016	0.022	-3.912	-4.135	-3.817
72	0.022	0.014	0.025	-3.817	-4.269	-3.689
96	0.023	0.016	0.029	-3.772	-4.135	-3.540
120	0.025	0.017	0.032	-3.689	-4.075	-3.442
144	0.026	0.018	0.031	-3.650	-4.017	-3.474
168	0.024	0.019	0.034	-3.730	-3.963	-3.381
192	0.025	0.021	0.032	-3.689	-3.863	-3.442
216	0.025	0.023	0.031	-3.689	-3.772	-3.474

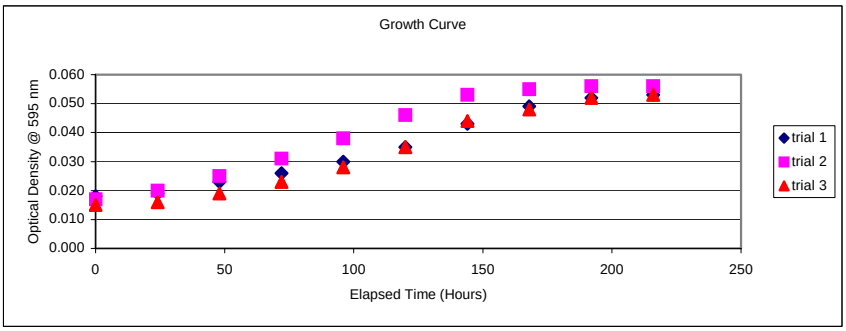


	Growth Rate
trial 1	0.0027
trial 2	0.003
trial 3	0.0049
Average	0.0035
STD	0.0012

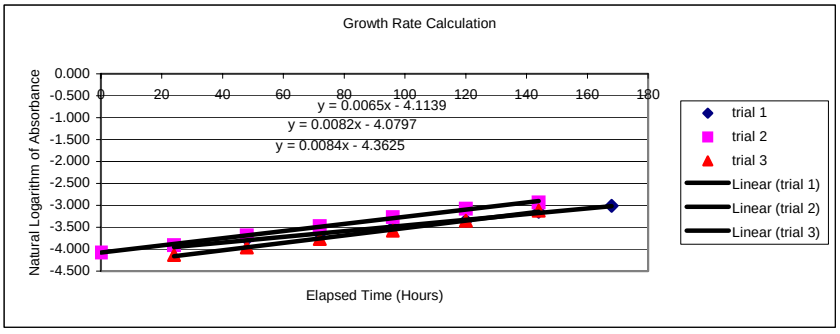


100 g/L

				logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.018	0.017	0.015	-4.017	-4.075	-4.200
24	0.019	0.020	0.016	-3.963	-3.912	-4.135
48	0.023	0.025	0.019	-3.772	-3.689	-3.963
72	0.026	0.031	0.023	-3.650	-3.474	-3.772
96	0.030	0.038	0.028	-3.507	-3.270	-3.576
120	0.035	0.046	0.035	-3.352	-3.079	-3.352
144	0.043	0.053	0.044	-3.147	-2.937	-3.124
168	0.049	0.055	0.048	-3.016	-2.900	-3.037
192	0.052	0.056	0.052	-2.957	-2.882	-2.957
216	0.053	0.056	0.053	-2.937	-2.882	-2.937

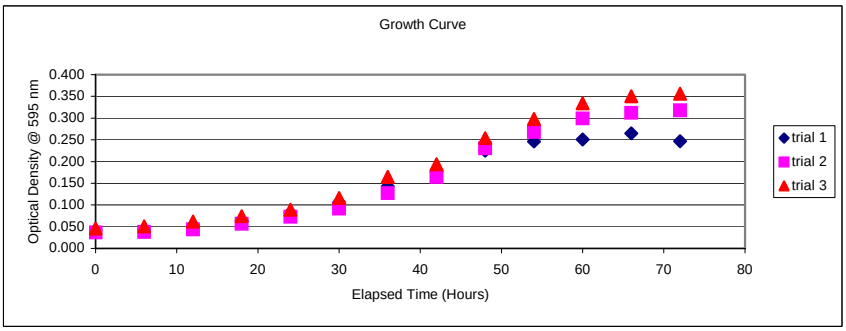


	Growth Rate
trial 1	0.0065
trial 2	0.0082
trial 3	0.0084
Average	0.0077
STD	0.0010

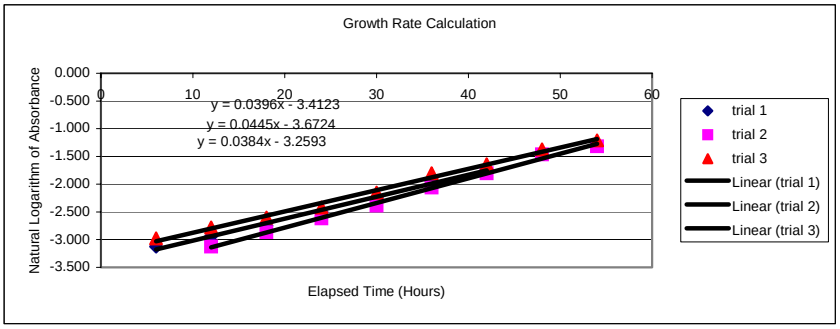


150 g/L

				logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.042	0.037	0.046	-3.170	-3.297	-3.079
6	0.044	0.038	0.051	-3.124	-3.270	-2.976
12	0.053	0.044	0.062	-2.937	-3.124	-2.781
18	0.064	0.057	0.074	-2.749	-2.865	-2.604
24	0.082	0.073	0.089	-2.501	-2.617	-2.419
30	0.106	0.092	0.116	-2.244	-2.386	-2.154
36	0.143	0.127	0.165	-1.945	-2.064	-1.802
42	0.176	0.165	0.194	-1.737	-1.802	-1.640
48	0.225	0.231	0.254	-1.492	-1.465	-1.370
54	0.246	0.268	0.298	-1.402	-1.317	-1.211
60	0.251	0.299	0.334	-1.382	-1.207	-1.097
66	0.265	0.312	0.350	-1.328	-1.165	-1.050
72	0.247	0.318	0.356	-1.398	-1.146	-1.033

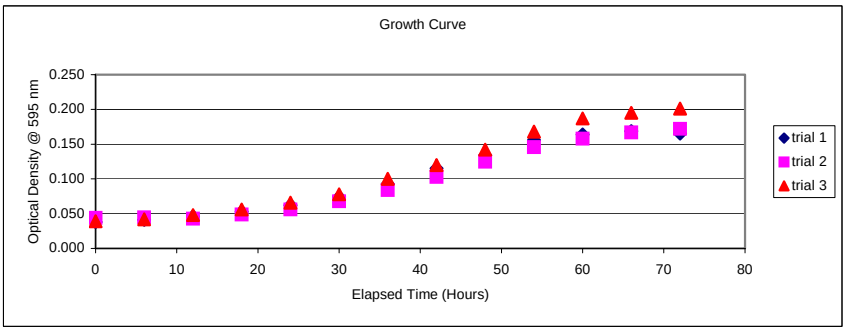


	Growth Rate
trial 1	0.0396
trial 2	0.0455
trial 3	0.0384
Average	0.0412
STD	0.0038

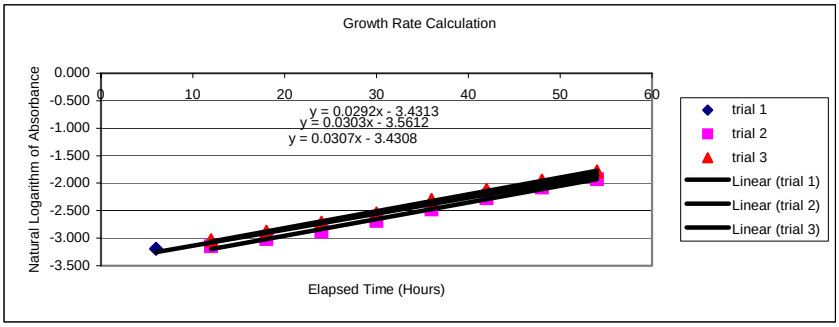


175 g/L

				logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.038	0.044	0.039	-3.270	-3.124	-3.244
6	0.041	0.045	0.042	-3.194	-3.101	-3.170
12	0.046	0.043	0.048	-3.079	-3.147	-3.037
18	0.053	0.049	0.056	-2.937	-3.016	-2.882
24	0.062	0.056	0.066	-2.781	-2.882	-2.718
30	0.074	0.068	0.078	-2.604	-2.688	-2.551
36	0.093	0.084	0.100	-2.375	-2.477	-2.303
42	0.115	0.103	0.120	-2.163	-2.273	-2.120
48	0.134	0.125	0.142	-2.010	-2.079	-1.952
54	0.156	0.146	0.168	-1.858	-1.924	-1.784
60	0.164	0.158	0.187	-1.808	-1.845	-1.677
66	0.169	0.167	0.195	-1.778	-1.790	-1.635
72	0.165	0.172	0.201	-1.802	-1.760	-1.604

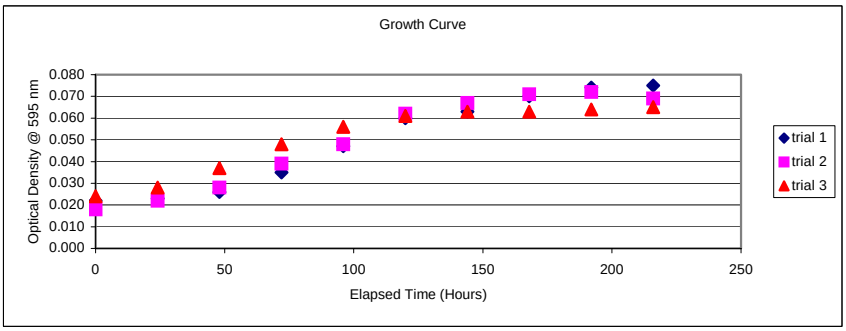


	Growth Rate
trial 1	0.0262
trial 2	0.0303
trial 3	0.0307
Average	0.0291
STD	0.0025

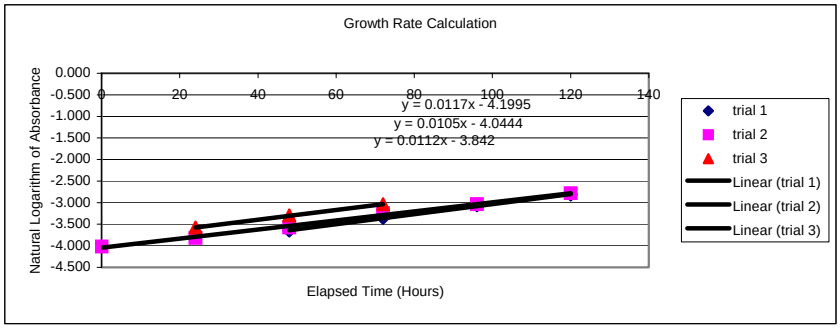


200 g/L

				logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.022	0.018	0.024	-3.817	-4.017	-3.730
24	0.023	0.022	0.028	-3.772	-3.817	-3.576
48	0.026	0.028	0.037	-3.650	-3.576	-3.297
72	0.035	0.039	0.048	-3.352	-3.244	-3.037
96	0.047	0.048	0.056	-3.058	-3.037	-2.882
120	0.060	0.062	0.061	-2.813	-2.781	-2.797
144	0.063	0.067	0.063	-2.765	-2.703	-2.765
168	0.070	0.071	0.063	-2.659	-2.645	-2.765
192	0.074	0.072	0.064	-2.604	-2.631	-2.749
216	0.075	0.069	0.065	-2.590	-2.674	-2.733

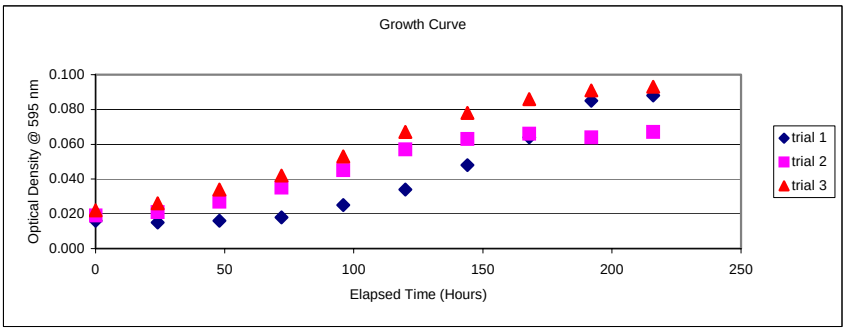


	Growth Rate
trial 1	0.0117
trial 2	0.0105
trial 3	0.0112
Average	0.0111
STD	0.0006

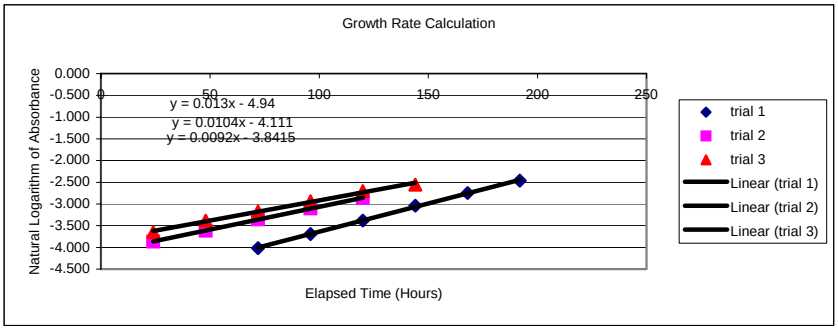


225 g/L

				logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.016	0.019	0.022	-4.135	-3.963	-3.817
24	0.015	0.021	0.026	-4.200	-3.863	-3.650
48	0.016	0.027	0.034	-4.135	-3.612	-3.381
72	0.018	0.035	0.042	-4.017	-3.352	-3.170
96	0.025	0.045	0.053	-3.689	-3.101	-2.937
120	0.034	0.057	0.067	-3.381	-2.865	-2.703
144	0.048	0.063	0.078	-3.037	-2.765	-2.551
168	0.064	0.066	0.086	-2.749	-2.718	-2.453
192	0.085	0.064	0.091	-2.465	-2.749	-2.397
216	0.088	0.067	0.093	-2.430	-2.703	-2.375

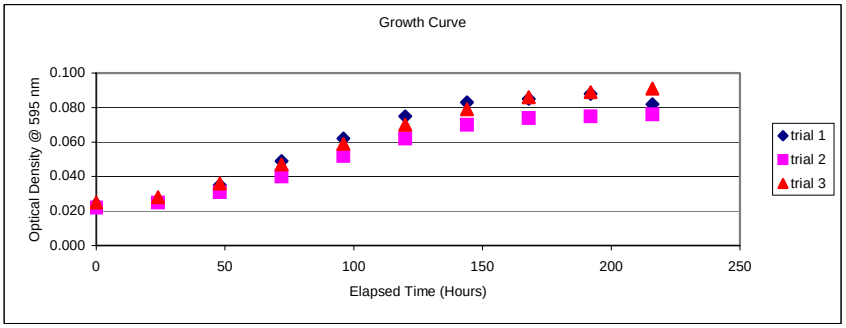


	Growth Rate
trial 1	0.013
trial 2	0.0104
trial 3	0.0092
Average	0.0109
STD	0.0019



250 g/L

				logarithms		
elapsed time	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
0	0.024	0.022	0.025	-3.730	-3.817	-3.689
24	0.026	0.025	0.028	-3.650	-3.689	-3.576
48	0.035	0.031	0.036	-3.352	-3.474	-3.324
72	0.049	0.040	0.047	-3.016	-3.219	-3.058
96	0.062	0.052	0.059	-2.781	-2.957	-2.830
120	0.075	0.062	0.070	-2.590	-2.781	-2.659
144	0.083	0.070	0.079	-2.489	-2.659	-2.538
168	0.085	0.074	0.086	-2.465	-2.604	-2.453
192	0.088	0.075	0.089	-2.430	-2.590	-2.419
216	0.082	0.076	0.091	-2.501	-2.577	-2.397



	Growth Rate
trial 1	0.0112
trial 2	0.0097
trial 3	0.0097
Average	0.0102
STD	0.0009