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Biological Volatile Organic Compounds (BVOCs) Emissions from the Planktonic diatom *Thalassiosira pseudonana*

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Abstract of the Thesis

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The sources and quantities of biological volatile organic compounds (BVOCs) are still a source of confusion in the scientific community. Phytoplankton and oceanic microbes have been considered insignificant contributors in the past as their role have only been investigated in depth in the last few years. The focus of this thesis was to examine the diatom species *Thalassiosira pseudonana* and determine the BVOC production which would occur over a time period of thirty days. This was done with a trap and purge system attached to a gas

chromatograph with flame ionization detector (Hewlett Packard GC-FID 6890). Two main compounds were observed: isoprene and dimethyl sulfide (DMS). After cell lysis had occurred, the production of BVOC either remained constant or increased dramatically. This study also addressed the effect that nutrient limitation could have on VOC emissions by *T. pseudonana*. Key nutrients such as phosphate, nitrate and silica were limited during incubations. Production of BVOC production decreased as nutrient limitation occurred. One secondary VOC, 2,3-dimethyl pentane (2,3-DMP), was observed as a by-product of environmental media. 2,3-DMP may be produced due to bacterial contamination or during the breakdown of vitamin B12 within the F/2nutrient media. Review of satellite measured chlorophyll-*a* ratio to BVOCs utilized in global calculations of BVOCs production suggest that the models may underestimate BVOC production because cell lysis is ignored.

Further studies of other oceanic plankton species must be done to gain understanding of their emissions of BVOCs and overall effects on atmospheric chemistry.

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Acknowledgments

"We are like dwarfs sitting on the shoulders of giants. We see more, and things that are more distant, than they did, not because our sight is superior or because we are taller than they, but because they raise us up, and by their great stature add to ours." John of Salisbury 12th century

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Introduction

Volatile organic compounds (VOCs) are released from a variety of sources, both by natural and anthropogenic processes. Anthropogenic VOCs are dominant in industrialized and heavily polluted areas. The use of certain materials such as paints and paint supplies, cleaners, pesticides/herbicides, building materials, glues and adhesives produce VOCs. Biogenic volatile organic compounds (BVOCs) are produced in pristine areas, although they also can have a significant influence on photo-smog episodes in densely populated areas (Schnitzler et al., 2005; Lewis et al., 2001). These episodes are related to the chemical reaction of anthropogenic NOx with emissions from local forests, such as in the cases of the Smokey Mountains of the US and the Himalayan Foothills haze (Ramanathan et al., 2005; Kang et al., 2000). VOCs can be highly reactive in the atmosphere and have short lifetimes, making them relevant to the chemistry of the lower troposphere (Carpenter et al., 1997). They also can serve as precursors to aerosol particles in the atmosphere (Hoffmann et al., 1997; Andersson-Skold and Simpson, 2001) as observed during low tide at Mace Head, Ireland and elsewhere (O'Dowd et al., 1998; Allen et al., 1999). Aerosols

have been shown to play a significant, but as yet undetermined, role in climate and weather changes by changing radiative forcing and causing precipitation suppression (Hutchison, 2003; Jayaraman, 2001). They also have been shown to lower visibility and to create health problems in animals (including humans) (Walker et al., 2000; Mitra et al., 2002). VOCs also degrade forming peroxy radicals which can form NO_2 from NO and lead to increases in local ozone (Helimig et al., 1996).

Biogenic volatile organic compounds were originally thought to include 300 different compounds (Graedel, 1979) including hydrocarbons, alcohols, and esters. More recently this list has expanded to include over a thousand compounds, including floral scents, gases from non-flowering plants, and other natural sources but the list still does not account for potentially thousands of compounds from many sources. The best studied and most important BVOCs in terms of concentration and reactivity within the atmosphere are methane, isoprene, the monoterpene family, dimethylsulfide (DMS), and ethylene. Methane, the monoterpene family, and ethylene are highly reactive with ·OH, NO_3, and other anthropogenic compounds, forming O_3 and aerosols which could affect the climate.

For example the reaction of isoprene with O_3 is known to produce methyl vinyl ketone and methacrolein (Aschmann et al., 1994). Isoprene is believed to

produce 1/3 of the secondary organic aerosol (SOA) and models suggest that by 2100 the current estimates of SOA production (12.2 Tg SOA yr^{-1}) will triple as result of increased biological contributions (Tsigaridis et al., 2007). There are however, many other less reactive BVOCs that are poorly understood. Estimates of total global emissions of BVOCs are uncertain and difficult to quantify for three principal reasons: (1) VOCs are still being identified, (2) identification is problematic due to sampling and analytical difficulties, and (3) short lifetimes after emission cause complications in determining concentrations and roles in the atmosphere. Chemical lifetimes of some BVOCs are very different and range from minutes (sesqiterpenes) to days (acetone) with shorter lifetimes during the night. BVOCs have shown variations in emissions due to temperature, light duration, intensity, environment and plant species.

Among the plants emitting VOCs are aquatic phytoplankton which account for 50% of global oxygen production. In the ocean, these single cell plants provide the foundation of the oceanic food web. Phytoplankton act as regulators of atmospheric conditions by fixing carbon dioxide. Charlson et al. (1987) speculated that phytoplankton could control cloud formation, and could theoretically cause cooling during global warming. However, very little is known about BVOC production by phytoplankton and the availability of nutrients which may have the potential to influence fluxes to the atmosphere.

Initial observations of isoprene production by terrestrial plants suggested that isoprene is produced continuously in daylight (Rasmussen, 1970). Terrestrial plants (which have been widely studied) show production of isoprene ranging from zero (European grape) to 172.9 μg $g(LDW)^{-1}h^{-1}$ (palm oil tree). Where LDW means leaf dry weight. Other plants produce mostly in the lower ranges of 1 to 2 μg $g(LDW)^{-1}h^{-1}$ for shrubs and 10-100 μg $g(LDW)^{-1}h^{-1}$ for various trees (Kesselmeier et.al., 1999). Phytoplankton have been observed to have a similar production cycle in light, and production rates within the lower range (<0.5 to 2 μg g $(LDW)^{-1}h^{-1}$) (LDW means leaf dry weight), but few studies have considered how fluxes might vary over the globe. This study was designed to examine the production of BVOCs by phytoplankton at different growth stages and how variations in the availability of key nutrients affect production rates and alter VOC emissions.

Background

Chohan (2002) noted that phytoplankton blooms have greatly decreased in the northern oceans over the last 20 years. Questions still abound as to why exactly this has occurred, and new questions have arisen: for example (1) what effects do phytoplankton blooms have on air quality near the coast where most major metropolises are situated throughout the world, and (2) whether these blooms are affecting air quality with the release of unidentified BVOCs into the environment .

Phytoplankton are known to release significant amounts of dissolved organic carbon (Cole et al., 1982; Baines and Pace, 1991) which can be photochemically oxidized to form VOCs which can be released into the atmosphere. Within a mesocosm Ratte et al. (1998) found quantities ranging from 0 to 180 pmol L^{-1} of dissolved organic carbon (DOC). Among the compounds produced in sea water containing phytoplankton were ethane, ethene, and isoprene. Field and laboratory experiments demonstrated variability in the chemical compounds present in culture media made from surface waters, depending on growth phase of diatoms and dinoflagellates. In addition to 'leaking' DOC during active growth, McKay et al. (1996) found non-methane

hydrocarbons including ethane, propane, and hexane in the media of axenic diatom and dinoflagellate cultures. During the dying phase of the same laboratory experiments, non-methane hydrocarbons also were found in the air above the cultures. These compounds were likely the result of oxidation of polyunsaturated lipids released into the culture medium following autolysis of the cells. Sartin et al. (2002) found a temporal pattern of VOC emissions from the coastal waters off Mace Head, Ireland, which was attributed to photo-dependent production associated with the degradation of biologically produced DOC in the seawater.

Fluxes of other volatile compounds including aromatics, alkenes, cycloalkanes, and terpenes (Bianchi and Varney, 1998) have been found to be governed by environmental factors such as time of day and season, cloud cover, wind direction, source of air mass, and temperature (Zhou and Mopper., 1990). Lewis et al. (1997) also found that compounds including isoprene, ethane, and propane showed strong diurnal variations with the greatest production at solar noon consistent with the timing of the greatest photosynthetic activity.

Two of the compounds studied extensively and observed as emissions from phytoplankton are isoprene and dimethylsulfide (DMS). Isoprene is a highly reactive compound which contributes to carbon monoxide and carbon dioxide production in the atmosphere (Doyle et al., 2004). The oxidation of isoprene can lead to an array of compounds like aldehydes, ketones, formaldehyde, and

nitrates which effect air quality (Carter and Atkinson, 1996; Ayers et al., 1997).The production of most BVOCs is dependent on temperature and light levels because these control photosynthesis, production of carbon and electron transport rates (Lamb et al., 1987; Sharkey et al. 1993). In the oceans, isoprene emissions were found to have a strong correlation to chlorophyll concentration with maximum emissions during summer and minimum during the winter, with total flux estimated as 0.38 Tg yr^{-1} global flux (Broadgate et.al., 1997). A recent oceanic isoprene model has produced fluxes between 0.31 and 1.9 Tg yr^{-1} by considering both a top-down and a bottom-up schematic (Arnold et. al., 2008).

Dimethylsulfide (DMS) is formed by enzymatic catalysis from dimethysulphoniopropionate (DMSP) and is produced along with acrylic acid (Challenger et al., 1959). This compound is thought to control the osmotic pressure of algal cells (Vairavamurthy et al., 1985). DMS has a lifetime of approximately a day or less within the atmosphere. It is believed that one half of earth's sulfur flux to the atmosphere comes from the ocean, with 90% being DMS (Andreae et al., 1984).

Thalassiosira pseudonana

Thalassiosira pseudonana is an adaptable fresh water and marine eukaryotic diatom and is known to bloom in many areas of the world during

spring to fall. For *T. pseudonana* the individual circular cell encasements are made of silica with valve like structures along the outer edge. *T. pseudonana* were the first diatoms to be genome mapped and are derived from a clone collected in 1958 from Moriches Bay (Long Island, New York) by Armbrust in 2004. This species grows well between temperatures of 10-30°C, with optimum growth at 17-21°C. Phytoplankton productivity is dependent on temperature, light availability (Ferguson et al. 1976), and nutrient availability. Important nutrients include nitrate, phosphate and silica (Takahashi and Parson, 1973).

Phytoplankton Conditional Variation

Nutrient limitation clearly has an effect on diatom productivity, particularly in the case of phosphate, nitrate, and silica. Goldberg et al. (1951) demonstrated a linear relationship between the size of diatoms and phosphate availability. Diatoms are poor competitors compared to other phytoplankton in low phosphate environments (Egge et al., 1998). Nitrate is also widely recognized as an important nutrient for growth (Carter et al., 2005; Parson and Takahashi, 1973; Egge and Aksnes, 1992). Phosphate and nitrate limited phytoplankton have lower growth rates and silica assimilation into cells is limited. Silica is important for test construction in diatoms like *T. pseudonana* (Egge and Aksnes, 1992). Silica limitation leads to lower carbon assimilation and faster sinking of diatoms

(Wong and Matear, 1999). Diatoms dominate within ocean environments whenever the silica concentrations are at least 2 μM.

The uptake of phosphate and nitrate are very similar (Perry, 1976) with the half saturation constant Kl, being lower for silica than for phosphate or nitrate (Parson and Takahashi, 1973). This suggests that phytoplankton can efficiently take up silica at lower concentrations than nitrate or phosphate, so phytoplankton will become limited by nitrate and phosphate than silica. If our initial assumption that emissions of VOCs are inversely dependent on growth is correct, then a lower production of VOCs should be seen during nutrient limitation which would be linked to limited growth rate.

Research objectives

The proposed study first was designed to examine what biological volatile organic compounds (BVOCs) are emitted from cultures of the phytoplankton *Thalassiosira pseudonana*. This was accomplished by sampling the phytoplankton cultures over a 30 day period with a purge and trap system. Using a gas chromatograph (Hewlett Packard 6890) with a flame ionization detector (FID), we can identify and quantify BVOCs. The second objective was to determine the affects of nutrient limitation (phosphate, nitrate and silica) on

BVOCs emission. This study was done to gain a better understanding of the controls on BVOC's production in nature which will affect the amount of exchange between the atmosphere and the ocean.

During cell lysis, volatile organic compound emissions may increase in the medium due to the breaking of cells. The organic material would be released from the cell interior and then would be able to photo-degrade. Variation in nutrient allotment may also cause VOC emissions to vary. Previous studies have observed the internal cell composition vary due to nutrient reservoirs, possibly affecting release of compounds on lysis.

A similar study was conducted by Shaw (2001). Five different species of plankton were studied: cyanobacteria (*Prochlorococcus mainus* and *Synechococcus*), *Emiliania huxleyi,* and the picoeukaryotes *Micromonas pusilla* and *Pelagomonas calceolate*. All species were found to produce isoprene, ranging from $1x10^{-21}$ to $4x10^{-19}$ moles $cell^{-1}$ day^{-1}. Production was related to size of cell as *E. huxleyi,* the largest cell, had higher production and *Prochlorococcus*, the smallest cell, had the lowest production. Sampling occurred during the first two weeks of growth, and spanned the growth, stationary, and lysis phase of the bloom. Isoprene production was constant during the exponential phase but decreased during stationary phase. However few samples were taken after cell

lysis occurred. In the present study samples were collected for at least four weeks to observe the decline of the phytoplankton cultures and its effect on BVOC production.

Water collection and Sample preparation

Sea water was collected at West Meadow Beach (Long Island, New York) in plastic carboys. Filtering was done in the laboratory with a .22µm MilliPak®40 Sterile Millipore cartridge to remove particulate organic matter. Aliquots of 400 mL were placed into 500 mL Erlenmeyer flasks. Standard stocks of F/2 nutrients (Table 1) were added to sea water in Erlenmeyer flasks which were then autoclaved at 250°F to kill bacteria. Then the media was cooled overnight. The following day trace metal nutrients were added, followed by 0.53 mL of a culture of the phytoplankton species *T. pseudonana*. Samples were placed into a walk-in cold room at 17°C, with a summer light cycle of 14 hours light and 10 hours dark provided by 40 Watt cool white light bulbs.

Study Procedure

Water was collected from a local inlet, filtered and autoclaved. Water was then amended with F/2 nutrient allotments and then inoculated with initially axenic phytoplankton culture, which was then grown for 30 days. Samples were not maintained axeically and some bacterial contamination could have occurred after inoculation. At various times, the cultures were purged to collect dissolved BVOCs onto a trap. This extraction technique was performed every five days after the first day of growth and cell production at each time point monitored by removing a 2mL aliquot from one of triplicate, samples used for purging. The samples of BVOCs were then eluted onto a gas chromatography for analysis.

Treatments

Different nutrient conditions were tested to observe whether nutrient limitation affected BVOCs production. Our *T. pseudonana* culture consisted of standard F/2 medium listed in Table 1. The three experimental cultures were prepared in such a way as to result in initial limitation of phosphate, nitrate or

silicate. The normal allotment of each nutrient was decreased by half of the normal amount used for F/2 for phosphate and nitrate, and to one fourth of the F/2 in the case of silicate. These four cultures were run in triplicate. Along with these samples, filtered sea water and filtered sea water with F/2 nutrients were monitored to determine if any volatile gases were emitted from the medium alone. Samples were taken on the first day of growth and every five days after.

Extraction and Pre-concentration

The extraction and pre-concentration method for BVOCs analysis is show in Figure 1. This system includes a hydrocarbon trap, two water traps, a 4 and 6 port valve connection system to the inline VOCs Tenex TA trap, a peltier cooler, temperature controller, a secondary pre-concentration loop trap, and a heated transfer line to the GC-FID. Before being bubbled into the phytoplankton sample as shown in Figure 2, a flow of extra dry air (50-80 mL) was passed through a Restek ® Hydrocarbon trap. This then passed into a glass water trap submerged in an ethanol and liquid nitrogen bath and kept at approximately -80ºC to remove any hydrocarbons and water vapor. The sample flasks were fitted with silicone stoppers with inlet and outlet ports. The glass traps submerged in the ethanol alcohol-liquid nitrogen bath were connected to the sample inlet port, which was a ¼ inch glass tube fitting that extended downward approximately half way into the

phytoplankton samples. This allowed approximately 4 Liters of gas to be bubbled through the sample, resulting in 95% removal of gases from samples of phytoplankton (Reimer et al. 2000). The outlet ports were ¼ inch glass tubing which extended out of the silicon stoppers in-line with an open tube water trap (Teflon tubing submerged into an ice-water bath). The second water trap was to sequester water vapor escaped the sample during the purging stage. The sampled air was then passed into the main pre-concentrating system containing the 4 and 6 port valves connected to the 10 inch long, ¼ inch in diameter Tenex TA trap, in-line with the GC-FID. These valves also control back-flush flow. The BVOCs in the sample are extracted on to the trap maintained at +3 to -4°C by peltier cooler, during the transmission of sampled air. The remaining gas flowed into a 10 Liter tank fitted with a pressure detector (strain gauge, measured in mbars) which determined volume collected.

Elution

Elution onto the GC commenced after sampling. The back-flush was turned to connect the pre-concentration inlet at the 4 port valve to ensure no other gases entered into the system. The injection was turned on at the 6 port valve to allow the GC-FID carrier gas (helium at a flow of 3 mL/min) to pass

through the Tenax TA trap leading to the GC-FID column. The trap was heated to 270-290°C with heating wire for 30 minutes to elute the BVOCs.

The sampled BVOCs were pre-concentrated on a 106 cm 0.32mm ID deactivated capillary tube from Alltech, which was looped five times and lowered into dewar of liquid nitrogen three minutes before collection. The liquid nitrogen was refilled twice during collection. This second pre-concentration was done to create a more precise sample injection for the GC-FID which improved compound peak clarity. During this second pre-concentration a GC-FID program was run on a laptop (Compaq Presario 2100), which collected data to ensure that no compounds were eluted onto the GC-FID column during this time.

Sample Analysis

The loops were removed from the liquid nitrogen dewar and placed into a warm water bath for two minutes at which point the sample was injected on the GC-FID column (30 meter, 0.25mm I.D. Restek MXT-624) . The oven program included an initial period of 5 minutes at 20°C, accomplished by placing dewar of liquid nitrogen within the oven. The oven was then ramped to 160°C at 6°C/min, and finally held for two minutes before returning to room temperature. Data was collected and examined using the program Chem Station by Agilent in the form of chromatographs as shown in Figure 3.

Additional measurements

After each purging, 2 mL of sample was extracted and pH was measured with pH testing strips (Quick Dip Aquarium Test Strip, pH of 6.2 to 8.8). These samples were placed into 20µL of Lugols and were used for cell counts with the use of a hemocytometer and a Nikon Labophoto-2 microscope.

Results

Cell counts

Over the thirty days of growth (720 hours), cell counts were found to increase for all treatments of phytoplankton (Figure 4). The ¼ silica limited treatments reached a maximum production at 120 hours with 3.73 x 10^8 cell/mL and then gradually decreased until the conclusion of the experiment. The control maximum (8.4x10^8 cell/mL), ½ nitrate limited treatment (4.5x10^8 cell/mL), and ½ phosphate (4.5x10^8 cell/mL) limited treatment peaked at 360 hours. The control and ½ nitrate limited treatment both experienced a dramatic decline to 6.8 x10^7 cell/mL and 7.5x10^7 cell/mL by 480 hours. The ½ phosphate limited treatment

declined more gradually. Overall the control sample with full nutrient allotment was observed to have both the greatest cell count increase and the greatest decrease compared to the three nutrient limited treatments. The pH of all treatments remained between 7.4 and 8.4 throughout the experiment, considered reasonable for this particular species (R. M. Kipp, 2007). All treatments initially began with a pH of 7.4 (hour 25), then increased to a pH of 8.4 at time point 120 hours. All treatments leveled off at a pH of 8.4 for a variable period of time before declining back to a pH of 7.8. The pH of silica depleted treatments declined at 360 hours. The control full nutrient treatments declined at 480 hours. Nitrate depleted treatments declined at 600 hours and the phosphate depleted treatments declined at 720 hours.

Removal of background signal

All data were initially recorded as area of peak measure in picoamps by seconds. With the use of a reference gas provided by Reimer lab from the University of Miami, the sensitivity of the GC for each compound was determined (Table 2). The reference gas was analyzed several times during the experiment to monitor any change in sensitivity that the instrument may have experienced. There was a maximum change of approximately 11.7% in sensitivity over the

course of the experiment. Each area was then multiplied by the average instrument sensitivity shown in Table 2 to convert area to the moles of compound collected during each purging treatment. The results of this are presented in Figures 5, 6, and 7 for isoprene, dimethylsulfide (DMS), and 2, 3-dimethylpentane (2,3-DMP). The F/2 was taken as the blank for both the isoprene and DMS net emissions are calculated by subtracting the blank from the individual phytoplankton treatment. The F/2 produced more 2, 3-DMP than the phytoplankton treatments resulting in negative emission rate. The variability between individual triplicate cultures is denoted by error bar on the average point within the figures, and is listed on the data tables.

Production Rate

Production rate was determined by dividing the change in the amount of BVOC in a treatment by the cell count of treatment and time between then and the last time point in hours.

Observations

The different nutrient treatments exhibited some variability in isoprene emission. Samples with ¼ silica concentration initially produced the greatest flux of isoprene at 5.26 x 10^{18} moles/cell/hr. The other treatments (½ phosphate and ½ nitrate) had lower maximum isoprene production of 1.87 x 10^{-19} and 4.84 x 10^{-20} moles/cell/hr respectively (Figure 8 and 9). Figure 9 contains the same time points as Figure 8 although the first time point for ¼ silica was removed to enlarge the graph for examination). 720 hours the control reached a peak production of 4.45 x 10^{-19} moles/cell/hr.(Figure 10,11,12,13 Table 3) We note that, isoprene production from the nutrient limited treatments, initial sampling at 25 hours became almost constant until hour 720. For the control treatment production was almost constant the entire time.

Initial fluxes of DMS in limited nutrient treatments (except for ½ phosphate) were higher than culture controls. (Figure 14 and Figure 15 contains the same time points although in Figure 15 the first time point for ¼ silica was removed to enlarge the graph for examination). The silica depleted culture had the greatest initial DMS flux (8.41 x 10^{-17} mole/cell/hr) followed by the nitrate depleted (5.55x 10^{-18} moles/cell/hr) and control (5.47 x 10^{-19} mole/cell/hr). An overall decrease of emissions of DMS occurred after the first purging for all of

treatments. The phosphate limited phytoplankton sample formed the least f DMS (2.36×10^{-19} mole/cell/hr) at 25 hours.

From 420 to 700 hours the control emissions decreased the most closely followed by silica and nitrate. During this time cell growth declined for all samples. The maximum production in the control culture occurred at 600 hours (2.24×10^{-17} moles/cell/hr), decreasing to 1.44×10^{-17} mole/cell/hr at 720 hours. The control culture produced more DMS than the other limited nutrient treatments. At 700 hours the silica limited culture (1.44×10^{-17} moles/cell/hr) was closest in production to the control, the nitrate limited culture (7.98×10^{-18} moles/cell/hr) followed and the phosphate limited culture (1.46×10^{-18} moles/cell/hr) produced the least DMS. Even considering the natural variability of the replicate samples, an overall trend is observed in all culture treatments of almost constant production from initial samples at 25 hours to 360 hour. Then an increase in production occurs from 360 to 720 hours. This increase coincided with the decline of cell production (Figure 16,17,18,19, Table 4).

The production of 2, 3-DMP within all phytoplankton treatments was observed to be lower than that of the F/2 nutrient treatment. 2, 3-DMP emissions were determined by subtracting results from F/2 medium alone from that of phytoplankton treatments. This created a negative net production of 2.3-DMP for all phytoplankton treatment as observed in Figure 20, and 21. (Figure 21

contains the same time points as Figure 20, although the first time point for ¼ silica was removed to enlarge the graph for examination). The net negative production may represent uptake of 2, 3-DMP by the cells in the treatments or lower production due to shading in the cultures. The control treatment (F/2 plus cells) was found to exhibit the greatest consumption of 2, 3-DMP at an average of $1.7x10^{-17}$ moles/cell/hr but also there was a high level of variability between the replicates. The ¼ silica limited treatment had the greatest average depletion at $7.5x10^{-18}$ moles/cell/hr and the least natural variability in the replicates (Figure 21). Overall the consumption or decline in production of 2, 3-DMP was greatest in the ¼ silica limited treatments, followed by the control treatments, ½ nitrate limited treatments, and finally the ½ phosphate limited treatment which consumed the least or produced the most 2,3-DMP. Triplicates of each treatment varied greatly at 25 hours (Figures 22, 23, 24, 25, Table 5). In the control sample, however, the last two time points of the sampling period had the greatest variability. Overall each treatment showed an almost constant consumption from 25 hours to 360 hours. After 360 hours a greater net uptake increase occurred.

Discussion

The two compounds we observed emitted directly from the phytoplankton were isoprene and diimethysulfide.

Isoprene

Shaw (2001) observed the VOCs emissions of five different organisms, including *E. huxleyi,* during the initial 14 days of their life cycle. The most prevalent VOC produced was isoprene, and production was strongly correlated to the cell size distribution between species. *E. huxleyi* was the largest cell, and it produced the greatest concentration of isoprene at 3.8×10^{-19} moles/cell/day. In the present study beyond the initial 14 days, approximately 1.07×10^{-17} moles/cell/day were produced by *T. pseudonana* on the 30th day of incubation. The average production during the entire incubation, excluding the initial peak, was about 3.83×10^{-18} moles/cell/day for *T. pseudonana*, which is approximately one-half the size of *E. huxleyi* . Shaw's chosen species of microbes were largely open ocean species rather than to diatoms which tend to be concentrated on the coastline. Previous studies have examined the dependence of nutrient availability

and growth rate on isoprene emissions (Goldberg et al., 1951; Egge et al., 1998; Carter et al., 2005), finding that the greater the dependence of growth on the particular nutrient, the greater the effect on isoprene emissions.

DMS

We observed an initial decrease in DMS emissions were observed between the 25th and the 120 hours, which was consistent with the results of Bucciarelli and Sunda (2003) who studied the effect of nutrient limitation on intercellular dimethylsulfoniopropionate (DMSP) (Figure 13 &14). Bucciarelli and Sunda (2003) also found that the nutrient limitation of nitrate, silica, and phosphate resulted in higher production of intercellular DMSP with cultures limited by nitrate having the greatest intercellular DMSP, followed by silica and phosphate limited cultures. Bucciarelli and Sunda (2003) found also relatively low production lasted until cell growth decreased at which time the intercellular DMSP began to grow exponentially. These researchers suggested that the increase in DMSP was due to limitation in nutrients which created a metabolic imbalance, thus inducing oxidative stress. This stress could affect the Calvin-Benson cycle causing programmed cell death and induced cell lysis. In our study

the control phytoplankton sample had the greatest cell production, and thus eventually greater cell death (Figure 4). The phosphate limited treatment on the other hand showed lower cell production. Probably due to the inability of cells to utilize ATP to form DNA, leading to less cell growth and DMS production. This is consistent in the cell growth over time in Figure 3.

Other experiments have also shown a relationship between decline in population and increase in DMS emission. A mesocosm experiment containing the diatoms *Skeletonema costatum* and *Thalasiosira nordenskioldii* found an increase of DMS after peak *chlorophyll a* concentrations had occurred (Restelli and Angeletti ,1993). These researchers suggested that the peak DMS emission occurred during senescence. Restelli et. al. (1993) found maximum concentrations of DMS follower by a rapid decrease. These works attributed the decrease to the presence of bacteria which could consume DMS in the mesocosm. They suggested that different concentrations of DMS could be attributed to three likely causes. First, the amount of chl-*a* in different species differs, thus causing an over or under estimation of species presence if chlorophyll is used to estimate phytoplankton cell number (Gibson et.al. 1990). Second, the zooplankton present in each mesocosm differed causing different levels of grazing. Third, the bacterial assimilation of DMS in each mesocosm

differed. We also observe natural variability as demonstrated by the uncertainty bars.

The implications of our final results support the possible importance of cell lysis. Cell lysis was considered to occur not only due to cell count decline but also to change in pH in which during uptake of CO_2 which increase sharply, and then a decrease sharply when cells die and organic matter remineralization. Our study showed, nutrient limitation of cultures led to the production of less DMS. This finding could suggest that in remote areas of the ocean where phytoplankton blooms develop and nutrient limitations occur, there could be higher emission DMS into the atmosphere.

2,3-DMP

We believed 2,3-DMP production could be from two sources, one being bacterial contamination, the other being photolysis of vitamin B_{12}. Bacterial contamination could have occurred at two times: during the addition of iron nutrients which occurs after autoclaving or during purging of samples. There is no literature of 2, 3 DMP production by bacteria, but due to their limited present they can not be discounted completely. 2,3-DMP may have been formed from the

photo-degradation of a compound contained in the F/2 nutrients. The only compound within the F/2 nutrients (table 1) which contained the molecular structure of 2, 3-dimethylpentane is that of vitamin B_{12}. Vitamin B12 is known to be unstable, but the decomposition products have not been identified in the marine environment. In sea water, vitamin B_{12} and others such as vitamin B_1 and B_6 are mainly produced by bacteria (Provasoli and Carlucci, 1976). In related phytoplankton studies D. Reimer (personal communication) also observed 2, 3-dimethylpentane.

Comparing the pattern of production of DMS in figure 13 and 14 to the production of 2,3-DMP in figures 19 and 20, we see there is a tendency for higher DMS production when low 2,3-dimethylpentane production and vice versa. The absolute amount produced of the two compounds also differed. For example at 600 hours, DMS production for the control was 1.79×10^{-18} moles/cell/hr while 2, 3-DMP exhibited net consumption of 1.00×10^{-18} moles/cell/hr (Tables 3). Methionine may control the rate of DMSP production (Hanson et.al., 1994) which in turn has been shown to control formation of DMS (Bucciarelli and Sunda, 2003). Methionine is enzymatic produced by vitamin B_{12} (Hanson et.al., 1994). In a study on vitamin B_{12} and iron co-limitation of phytoplankton in the Ross Sea, Bertrand et al.(2007) found the nutrient (phosphate, nitrate, and silica) depleted phytoplankton took up more vitamin B_{12} and iron compared to control

phytoplankton samples, and the effect was largest if silica was depleted This could explain why the silica limited samples created the lowest production of 2,3-dimethylpentane or greatest uptake of vitamin B_{12}.

Model implications

Models used to examine global isoprene emissions have concentrated on phytoplankton species, nutrient availability, and variables such as light intensity in combination with remote sensing of chlorophyll-*a* (Palmer and Shaw 2005; Arnold et al., 2008). These models have estimated net fluxes to the atmosphere from the ocean of 0.31 ±0.08 to 1.9 Tg/yr. These modeld are based on have correlation of chlorophyll-*a* to isoprene emissions in cultures which have been interpreted to suggest that there is a constant production of isoprene per unit chlorophyll-*a*, although the value may vary between species. Using observations a correlation between light intensity and isoprene production, and using a model Gantt et al. (2009) predicted global production rates of approximately 0.92 Tg/yr isoprene production. However their field data also found higher production of isoprene on certain days with lower light production, and occurrence of greater production on day with low chlorophyll-a counts. In these models the authors

used laboratory data of isoprene fluxes from different cultures, but only included data from day 1 to day 14, or until the beginning of bloom decline to determine the isoprene/chl-*a* relationship. Our studies have shown that during bloom decline, or cell lysis that production of isoprene per cell is relatively constant although cell numbers are declining. During cell lysis isoprene would increase and models would underestimate production of isoprene. If this pattern occurs with other species, this effect may explain the discrepancies between observed model predictions which large uncertainties (±40-50% and *in situ* observations.).

Models of DMS production mainly focus on estimations of DMSP production and conversion to DMS (Kloster et al., 2007). This approach attempts to account for the variations in DMSP production of different phytoplankton species (Kettle et al., 1999). The dataset, which is a collaborative effort of several laboratories, produced a range of estimates of DMS fluxes (from 15 to 33 Tg S yr^{-1}), with an uncertainty of ±50% in global DMS emissions (Kettle et al., 2000). One of the most recent assessment of DMS models found maximum emissions were occurring during a 'summer paradox', where the DMS maximum concentration occurred after the greatest phytoplankton cell production or highest chlorophyll counts (Vezina et.al., 2007). These findings are consistent with our research where we found that it was only after cell growth began to decline that DMS flux decreased.

Conclusions

The purpose of this research was to observe the production of volatile organic compounds from the diatom, *Thalassiosira pseudonana*. Following the work of Shaw (2001), we wanted to examine production of VOCs during the height of growth, but also later when cell lysis had begun. We postulated that the greatest VOCs emission could occur during cell death due to greater dissolved organic compounds production during this time. We also looked at the effect of nutrient variability on VOCs production. Our curiosity about production of these emissions springs from the past and recent scientific community investigations into the contributions of phytoplankton productivity to atmospheric chemistry.

Previous studies have ranged from understanding precursors of atmospheric emissions to cloud formation to secondary organic aerosol production, and the impacts of theses on climate forcing and air quality. Human and natural influences, such as urban run off and hurricanes which can enhance lifetimes of blooms or extend them to areas of the ocean once considered deserts could have large impacts on gas emissions. This research area is not only important to our current understanding of atmospheric chemistry over the

remote ocean, but could contribute to our greater f ability to predict climate changes.

We observed two compounds produced during the life cycle of the phytoplankton, isoprene and DMS. We found that the isoprene was produced at an almost constant rate over the duration of the experiment The DMS initially occurred at a constant production rate but after approximately 360 hours the rate increased in all samples coincident with population collapse as indicated by cell per milliliter decrease denoting in contrast. The control treatments containing F/2 nutrients experienced the greatest production followed by ¼ silica limited treatments, ½ nitrate limited treatments, and ½ phosphate limited treatments. This is in agreement with our initial hypothesis of DMS production being dependent more on cell death than on of cell growth. Both isoprene and DMS emission production varied due to nutrient limitation.

We also observed the production of volatile organic compounds not emitted by the phytoplankton, but which rather were produced in the filtered and autoclaved sea water nutrient media. The most abundant VOC was 2, 3-dimethylpentane. These emissions are likely to be the result of production by contaminating bacteria, or vitamin B_{12} photolysis. 2, 3-DMP was found to have its greatest production in the nutrient enriched media.

This study has increased our understanding of volatile organic compound production within the environment, but it has also generated new questions. Plans to further delve into this area of cell lysis and VOCs production will lead us into aerosol production eventually. The next step will be to verify our results with other phytoplankton species, and in sunlight conditions instead of white light which most likely will increase VOCs production. The data from this study also may be useful to further improving isoprene and DMS models. We are currently looking into the relationship between chlorophyll concentrations and VOCs production. VOC/chl-*a* may be different during exponential growth than during cell lysis which would account for apparent inconsistencies in these models. Another area we are investigating will be possible vitamin breakdown and VOC production.

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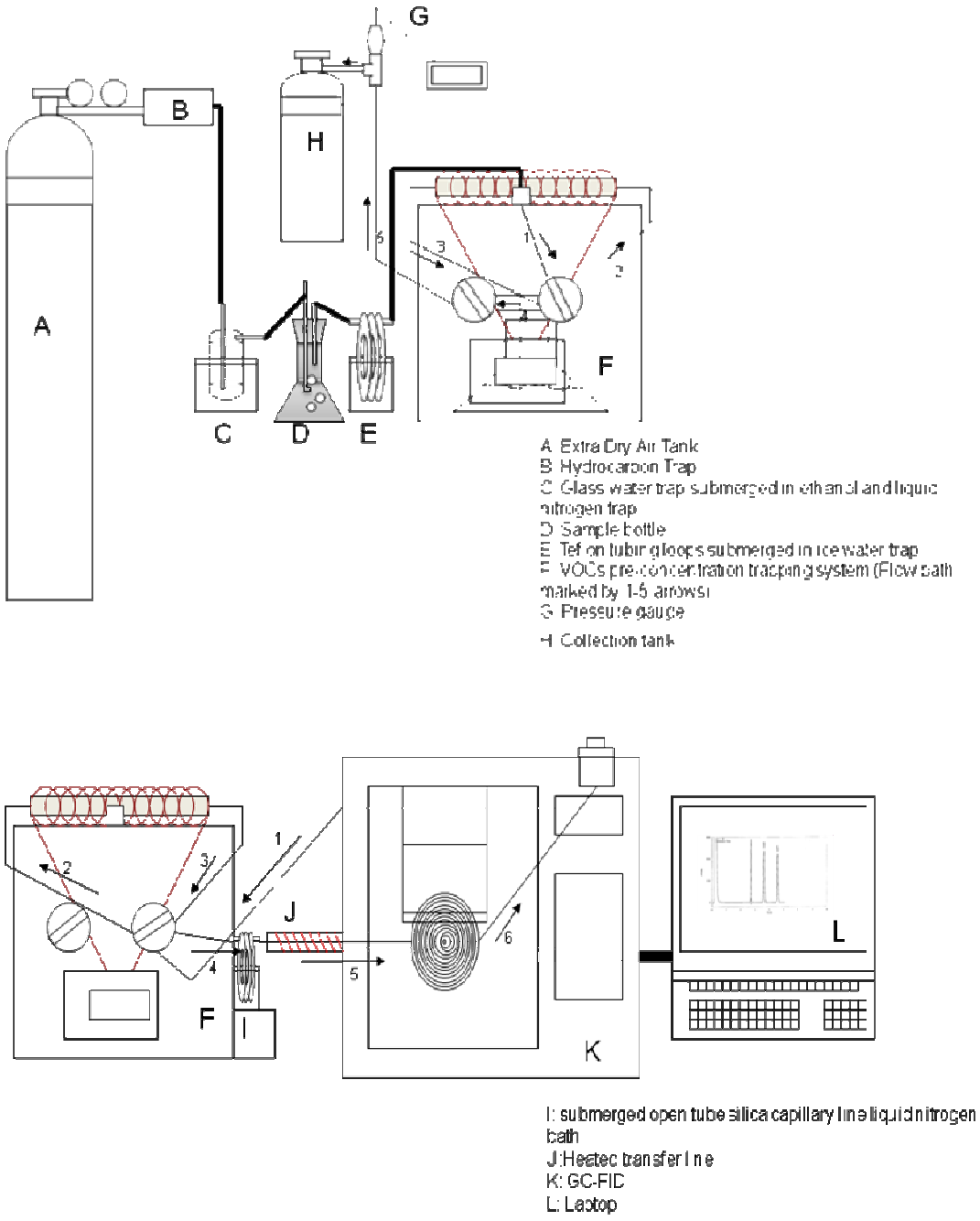

Figure 1

Schematic of Instrumentation for extraction and pre-concentration

Figure 2

Storage apparatus for phytoplankton through out the experiment, Erlenmeyer flask with silicone stopper (inlet and outlet glass tubing)

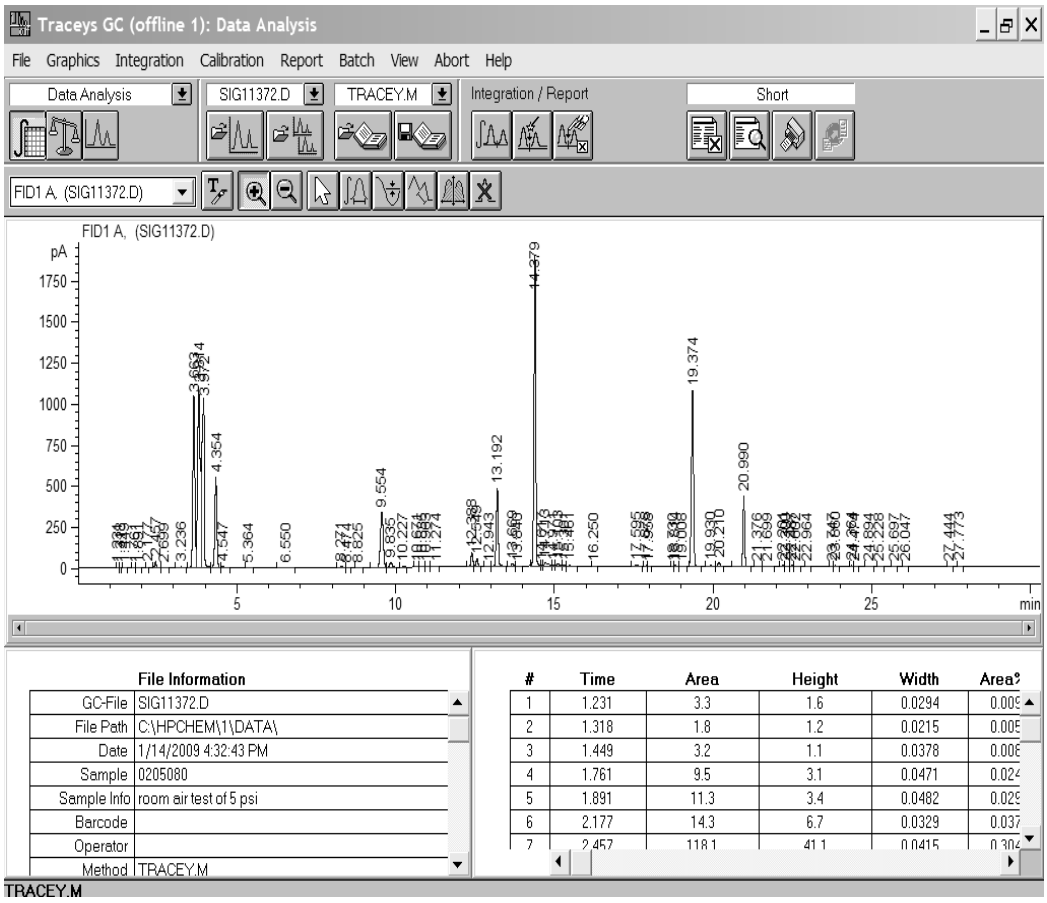

Figure 3

Example of data collected with the lab view program

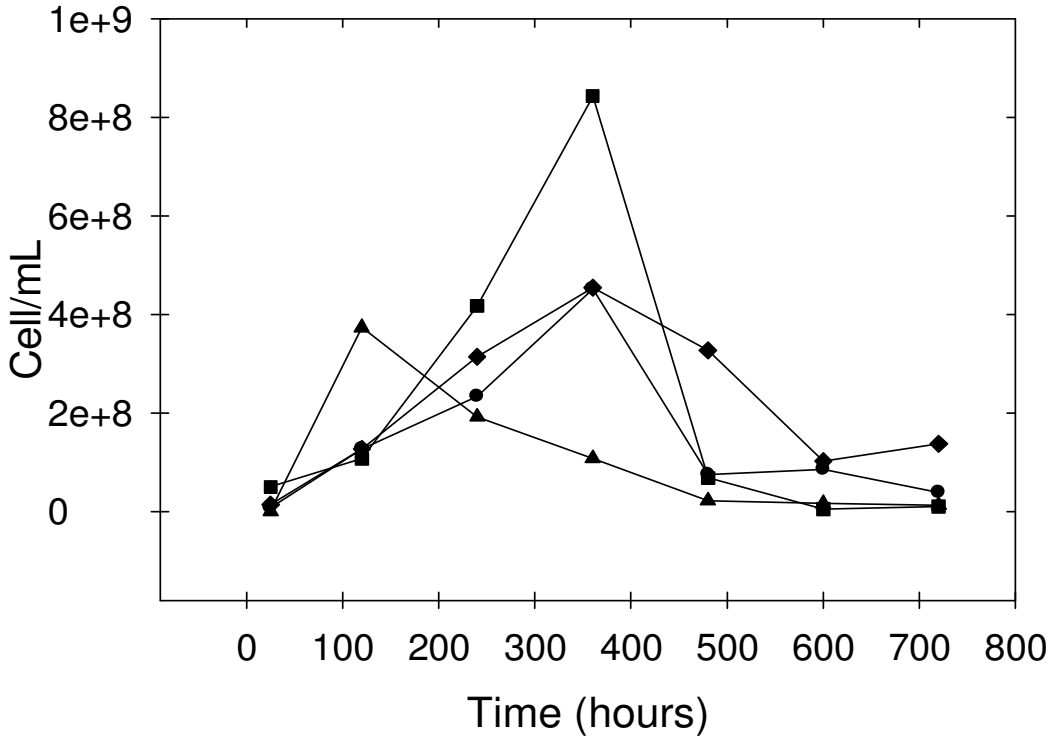

Figure 4

Cell growth monitored over thirty day experiment. Samples of control (full nutrient treatments) (squares), ½ phosphate nutrient treatments (diamonds), ½ nitrate nutrient treatments (circles), and ¼ silica treatments (triangles)

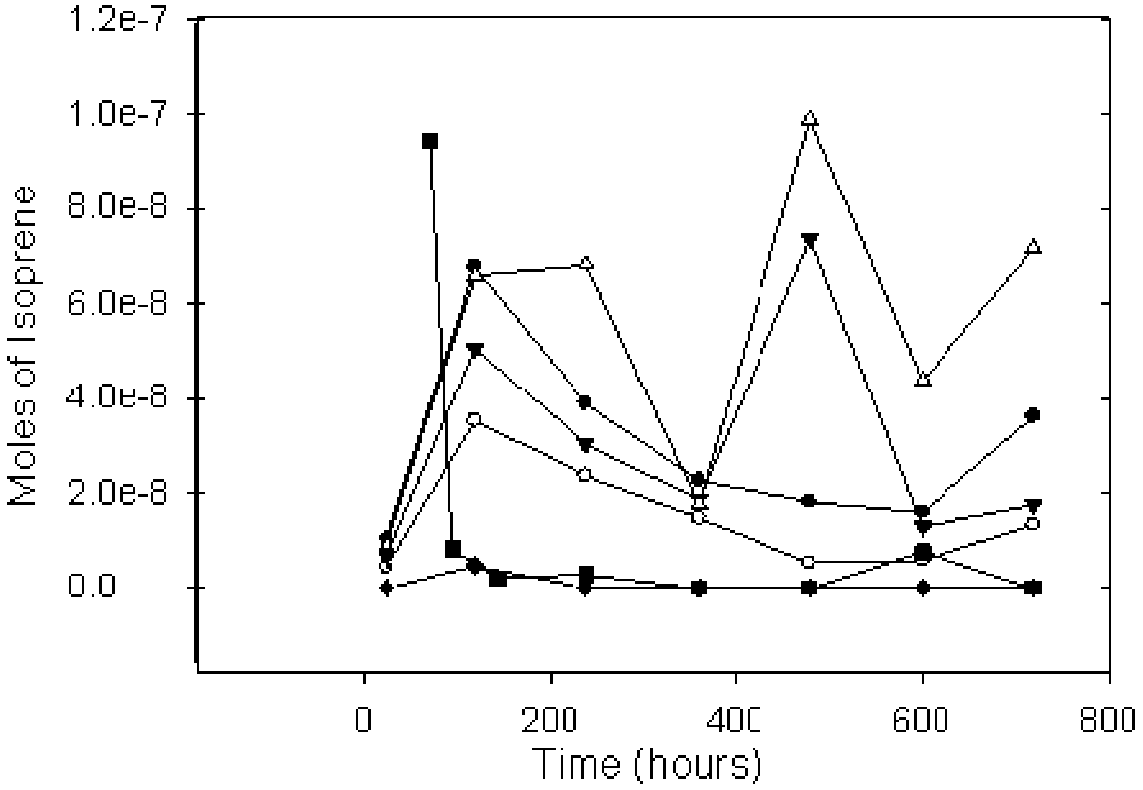

Figure 5

Moles of isoprene produced during each purging over the 30 day sampling period. Samples of control (full nutrient treatments) (closed circles), ½ phosphate nutrient treatments (open circles), ½ nitrate nutrient treatments (downward closed triangles), and ¼ silica nutrient treatments (open triangles)), sterilized filtered sea water (squares) and sterilized filtered seawater with F/2 full nutrient treatments (diamonds)

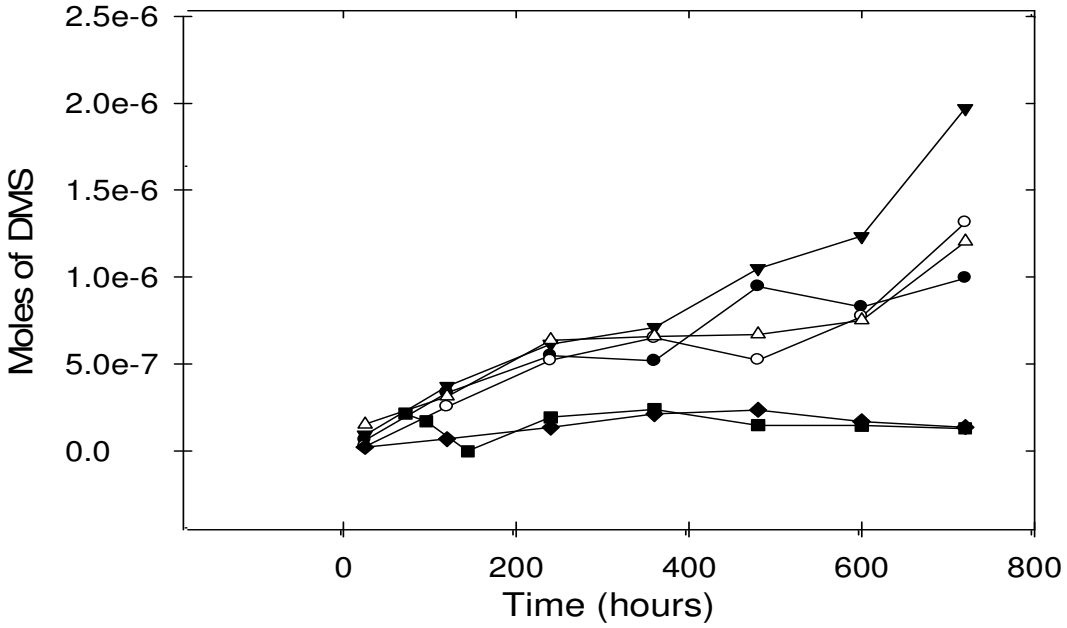

Figure 6

Moles of DMS produced during each purging over the 30 day sampling period. Samples of control (full nutrient treatments) (closed circles), ½ phosphate nutrient treatments (open circles), ½ nitrate nutrient treatments (downward closed triangles), and ¼ silica nutrient treatments (open triangles)), sterilized filtered sea water (squares) and sterilized filtered seawater with F/2 full nutrient treatments (diamonds)

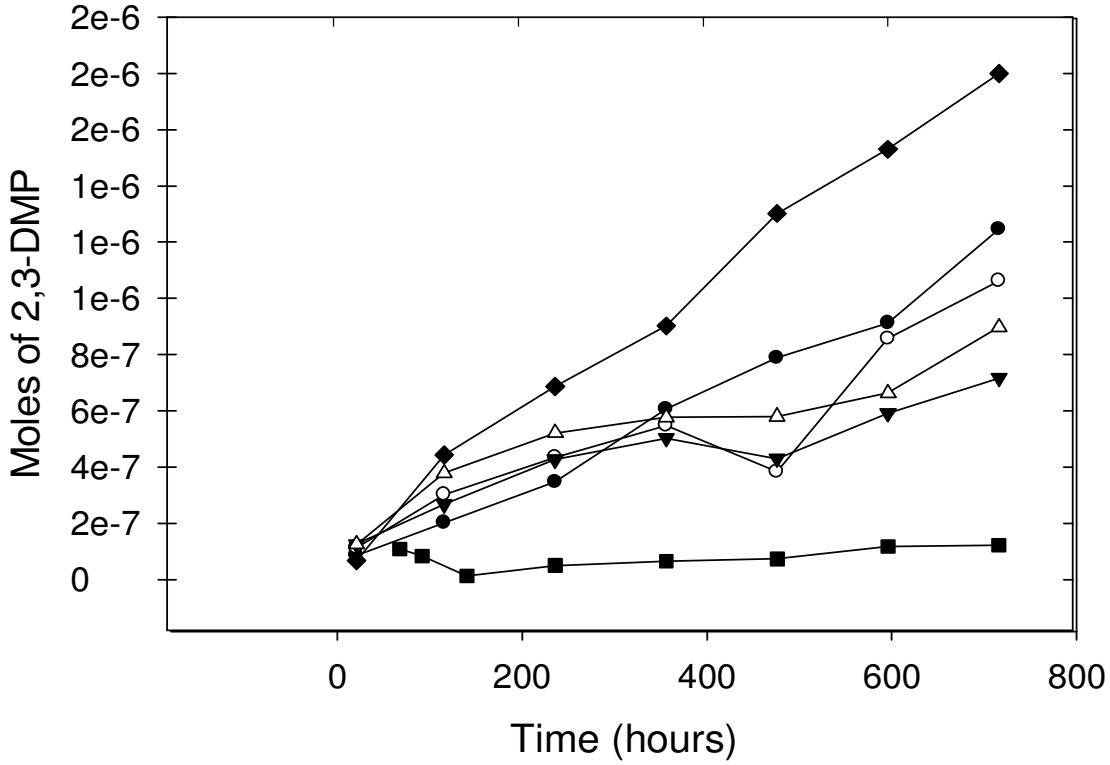

Figure 7

Moles of 2,3-DMP produced during each purging over the 30 day sampling period. Samples of control (full nutrient treatments) (closed circles), ½ phosphate nutrient treatments (open circles), ½ nitrate nutrient treatments (downward closed triangles), and ¼ silica nutrient treatments (open triangles)), sterilized filtered sea water (squares) and sterilized filtered seawater with F/2 full nutrient treatments (diamonds)

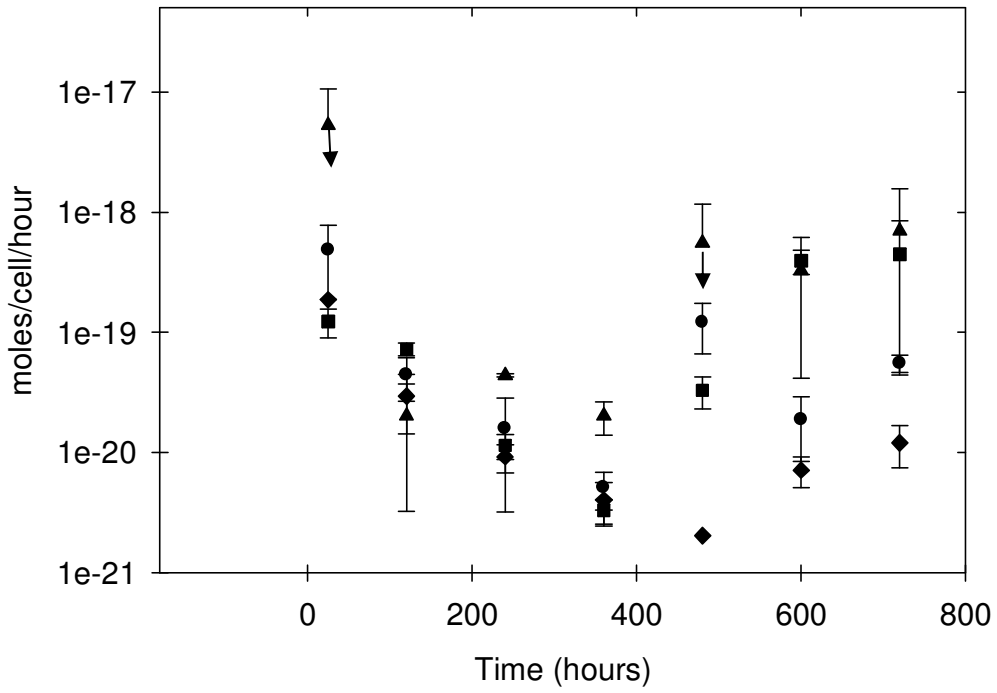

Figure 8

Average fluxes of isoprene produced in mole/cell/hour over the 30 day period. Samples of control (full nutrient treatments) (squares), ½ phosphate nutrient treatments (diamonds), ½ nitrate nutrient treatments (circles), and ¼ silica treatments (triangles). Points with arrows contain error bars which extend pass the graph area.

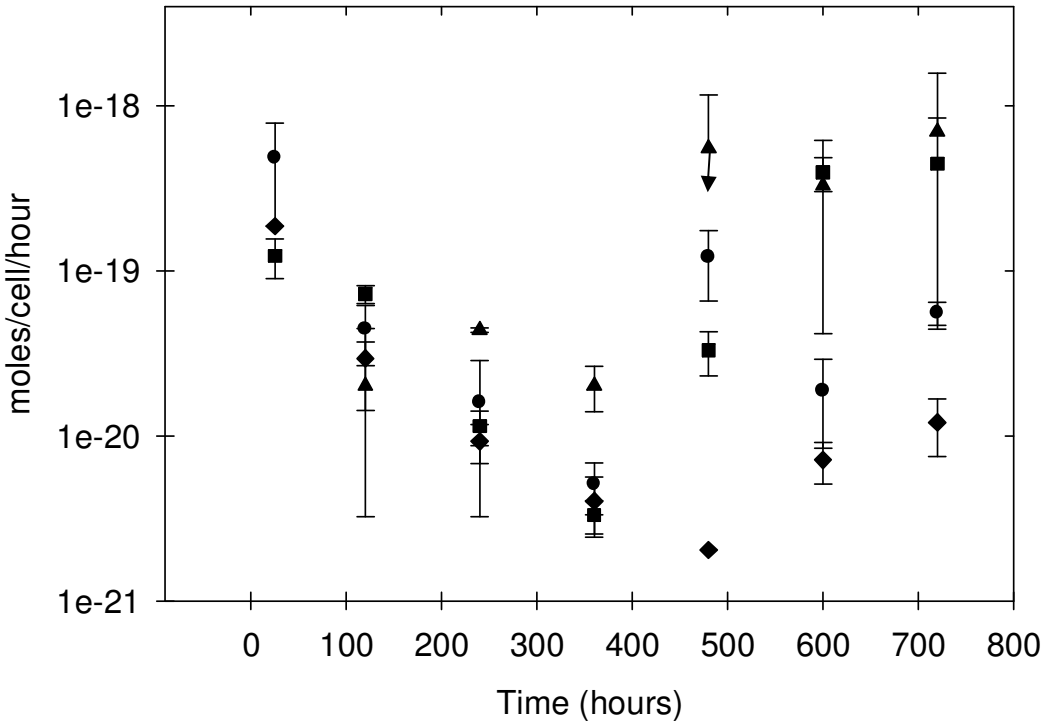

Figure 9

Average fluxes of isoprene produced in mole/cell/hour over the 30 day period with the removal of observation one of silica at 25 hour incubation. Samples of control (full nutrient treatments) (squares), ½ phosphate nutrient treatments (diamonds), ½ nitrate nutrient treatments (circles), and ¼ silica nutrient treatments (triangles).

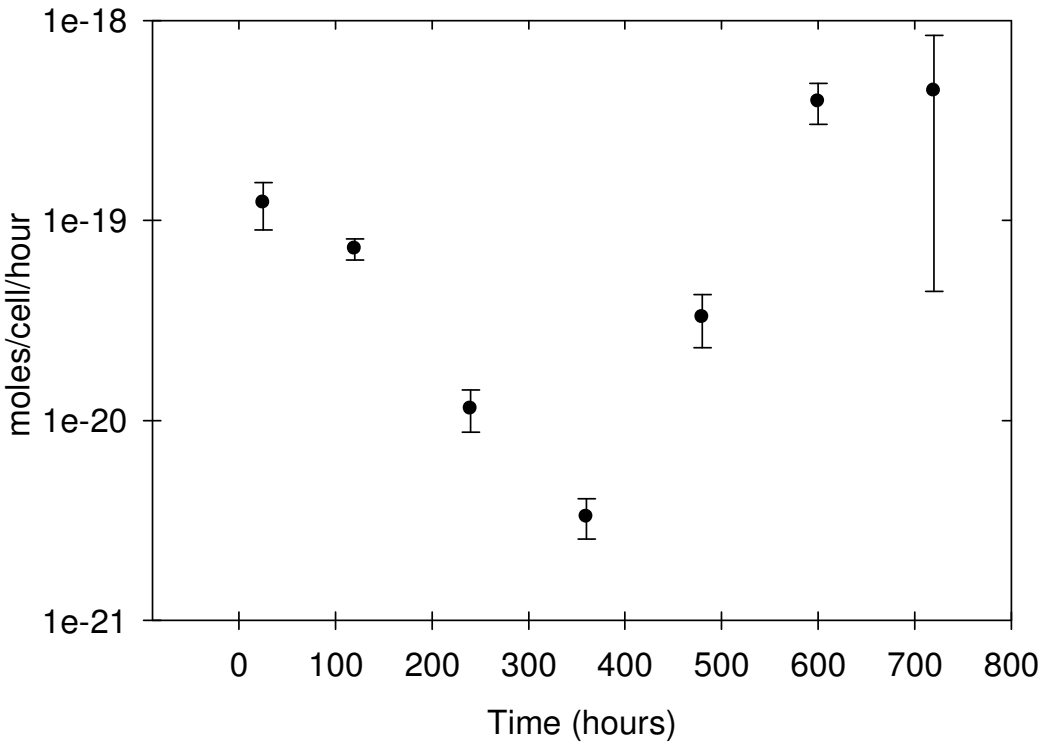

Figure 10

The natural variation within the triplicate samples of isoprene flux production in moles/cell/ hr over time of the control (full nutrient treatments)

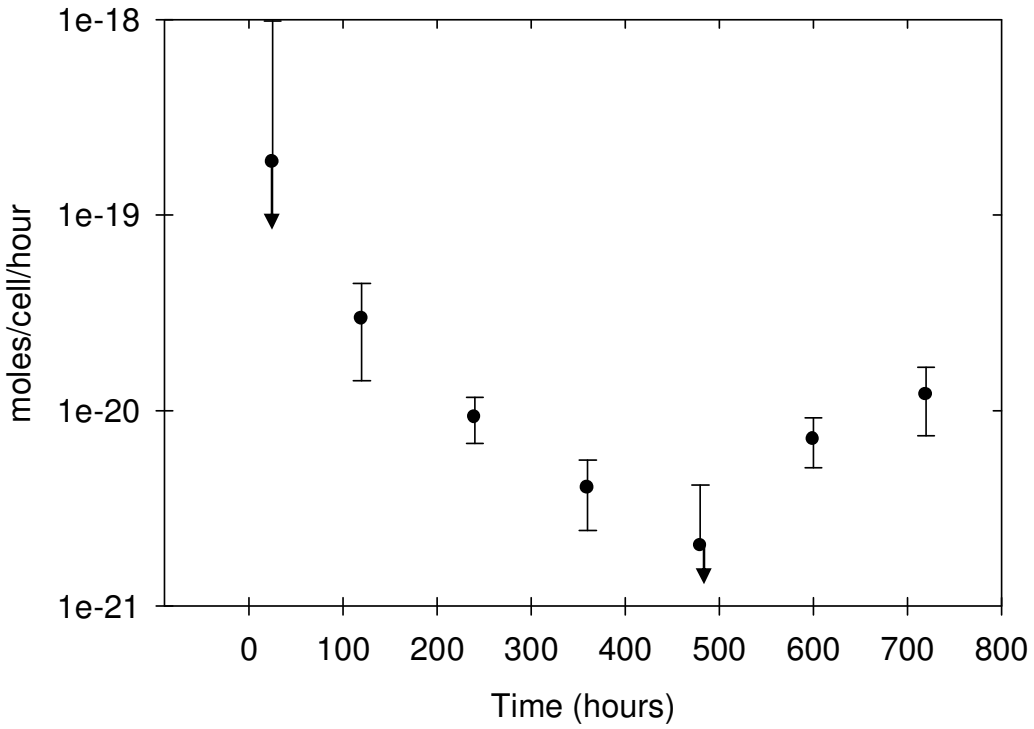

Figure 1

The natural variation within the triplicate samples of isoprene flux production in moles/cell/ hr over time of ½ phosphate nutrient treatments. Points with arrows contain error bars which extend pass the graph area.

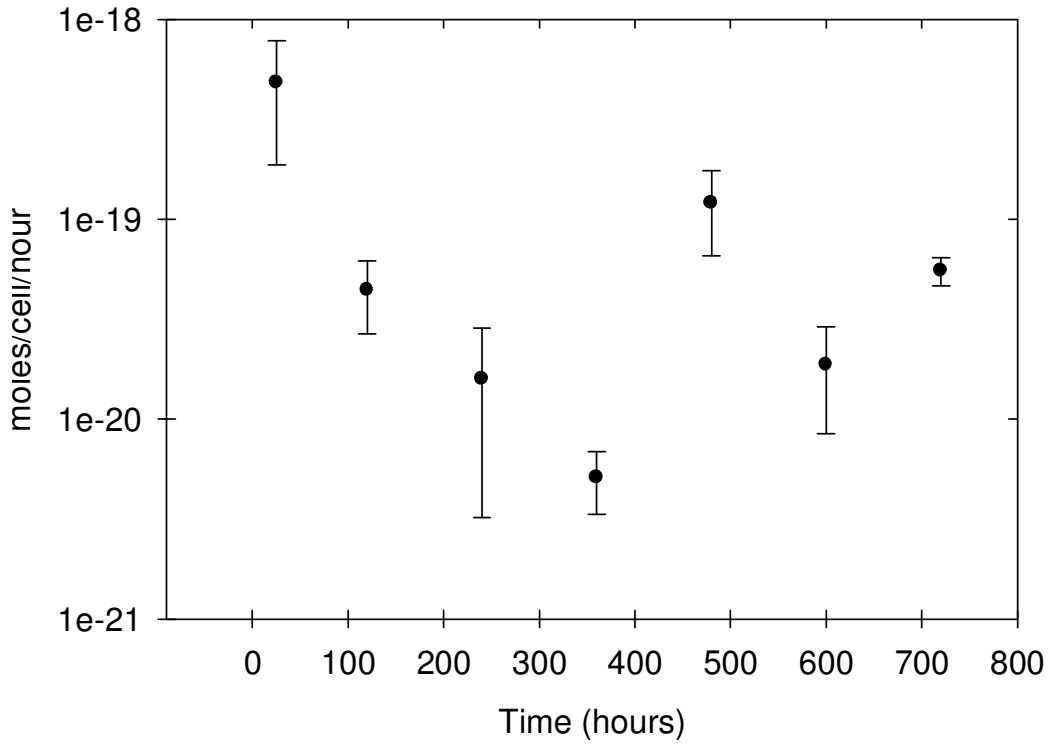

Figure 12

The natural variation within the triplicate samples of isoprene flux production in moles/cell/ hr over time ½ nitrate nutrient treatments

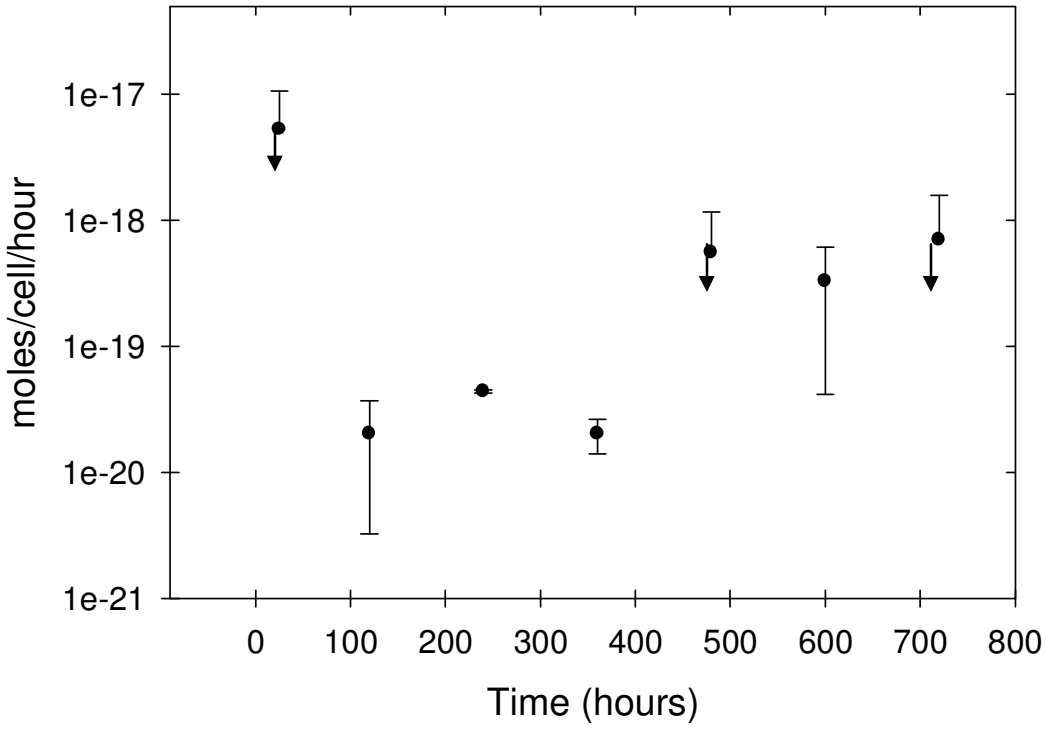

Figure 13

The natural variation within the triplicate samples of isoprene flux production in moles/cell/ hr over time of ¼ silica nutrient treatments. Points with arrows contain error bars which extend pass the graph area.

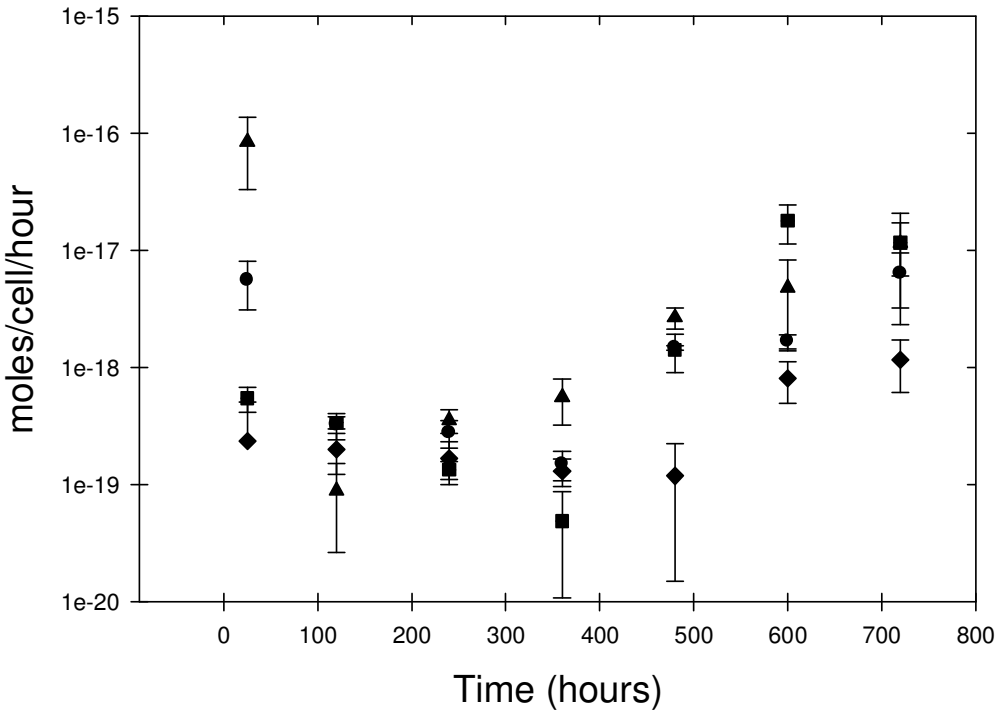

Figure 14

Average fluxes of dimethyl sulfide (DMS) produced in mole/cell/hour over the 30 day period. Samples of control (full nutrient treatments) (squares), ½ phosphate nutrient treatments (diamonds), ½ nitrate nutrient treatments (circles), and ¼ silica treatments (triangles)

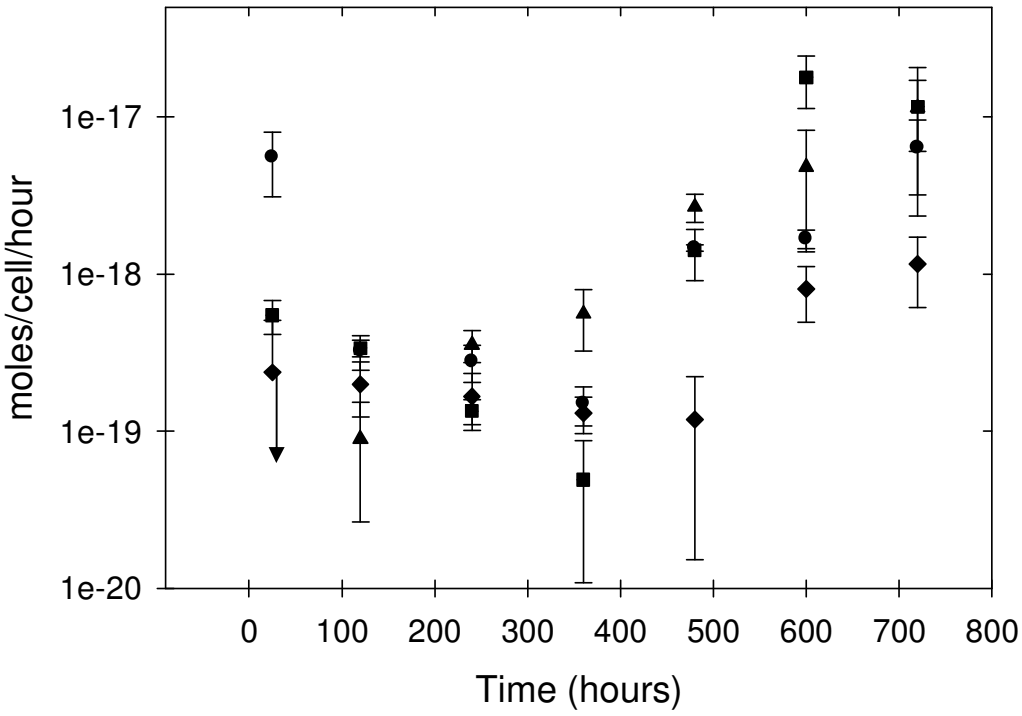

Figure 15

Average fluxes of dimethyl sulfide (DMS) produced in mole/cell/hour over the 30 day period with the removal of observation one of silica at 25 hour incubation. Samples of control (full nutrient treatments) (squares), ½ phosphate nutrient treatments (diamonds), ½ nitrate nutrient treatments (circles), and ¼ silica treatments (triangles). Points with arrows contain error bars which extend pass the graph area.

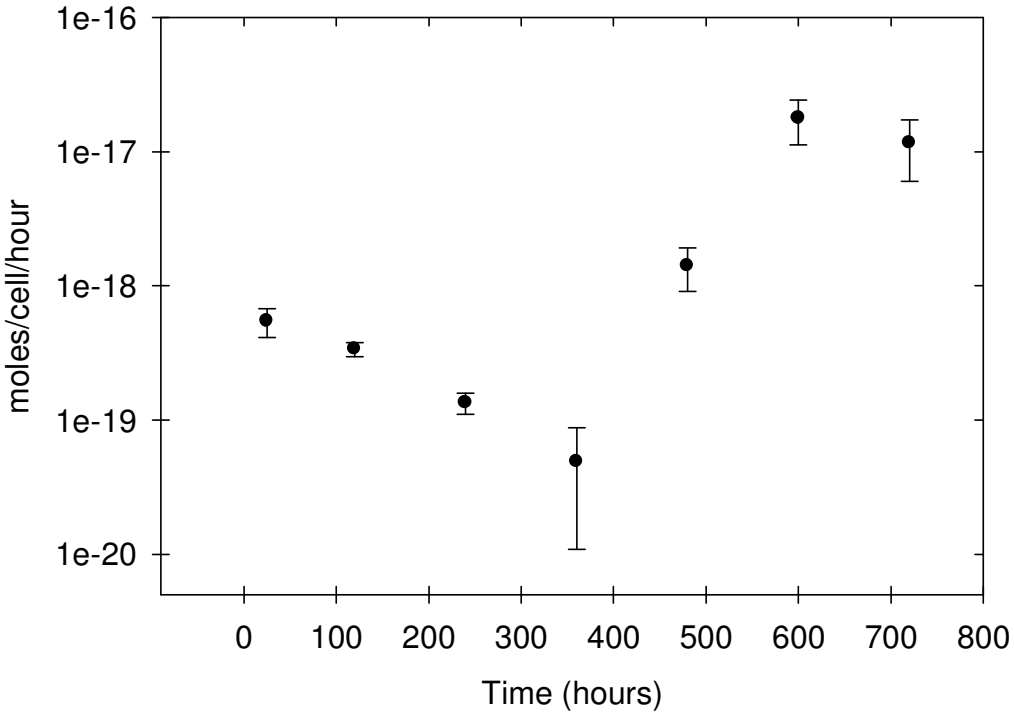

Figure 16

The natural variation within the triplicate samples of dimethyl sulfide (DMS) flux production in moles/cell/ hr over time of the control (full nutrient treatments).

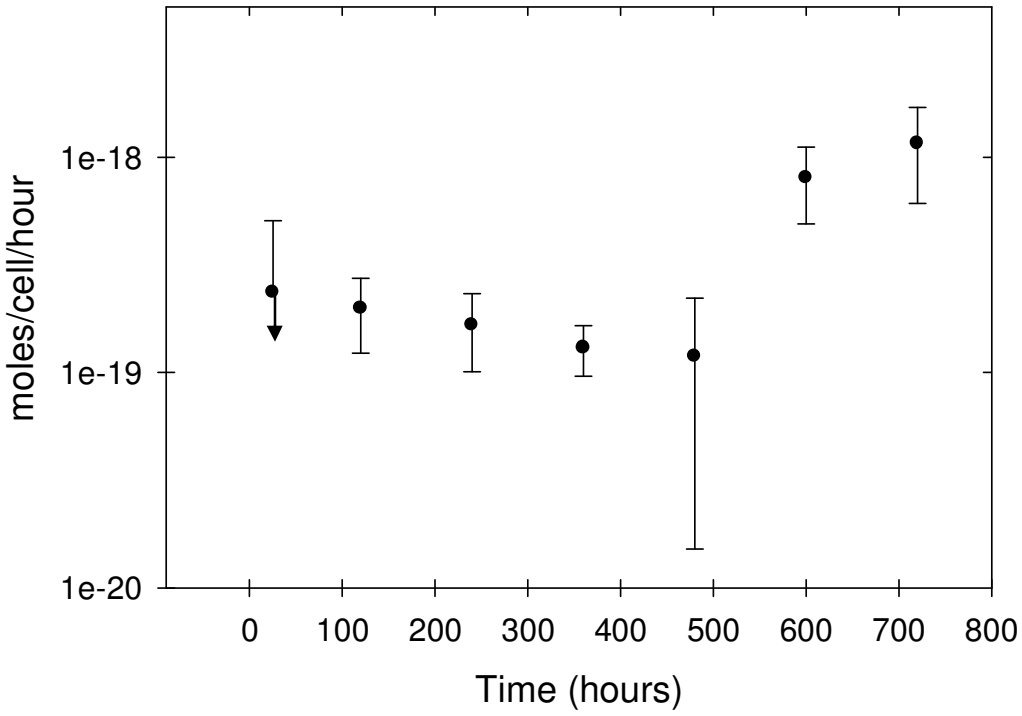

Figure 17

The natural variation within the triplicate samples of dimethyl sulfide (DMS) flux production in moles/cell/ hr over time of the ½ phosphate nutrient treatments. Points with arrows contain error bars which extend pass the graph area.

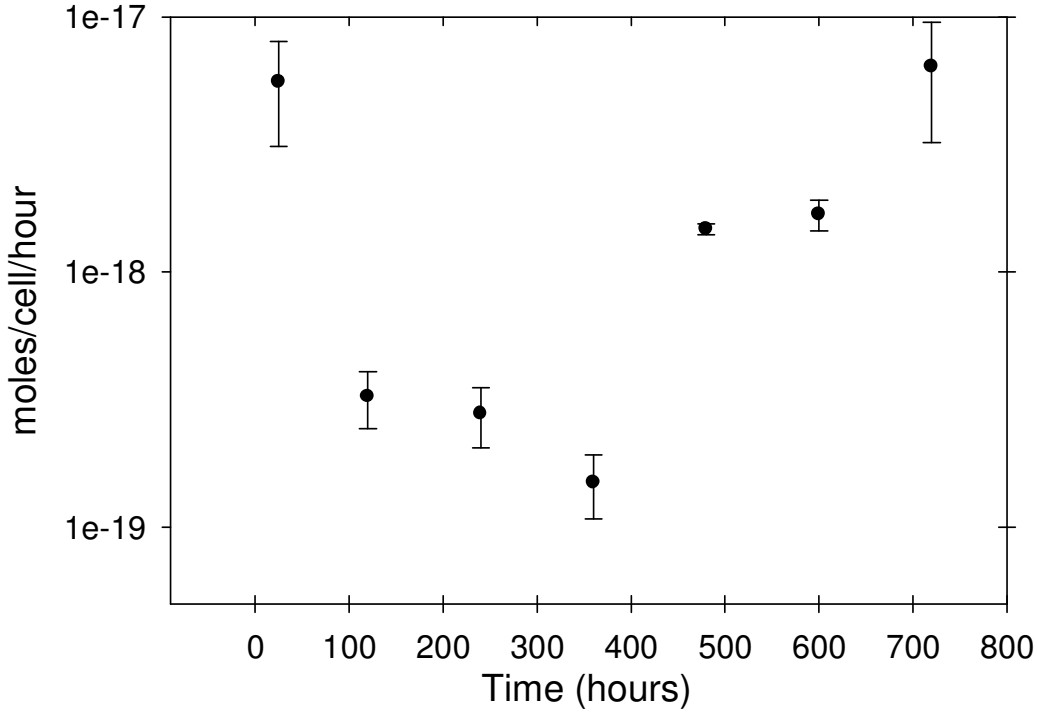

Figure 18

The natural variation within the triplicate samples of dimethyl sulfide (DMS) flux production in moles/cell/ hr over time of the ½nitrate nutrient treatments.

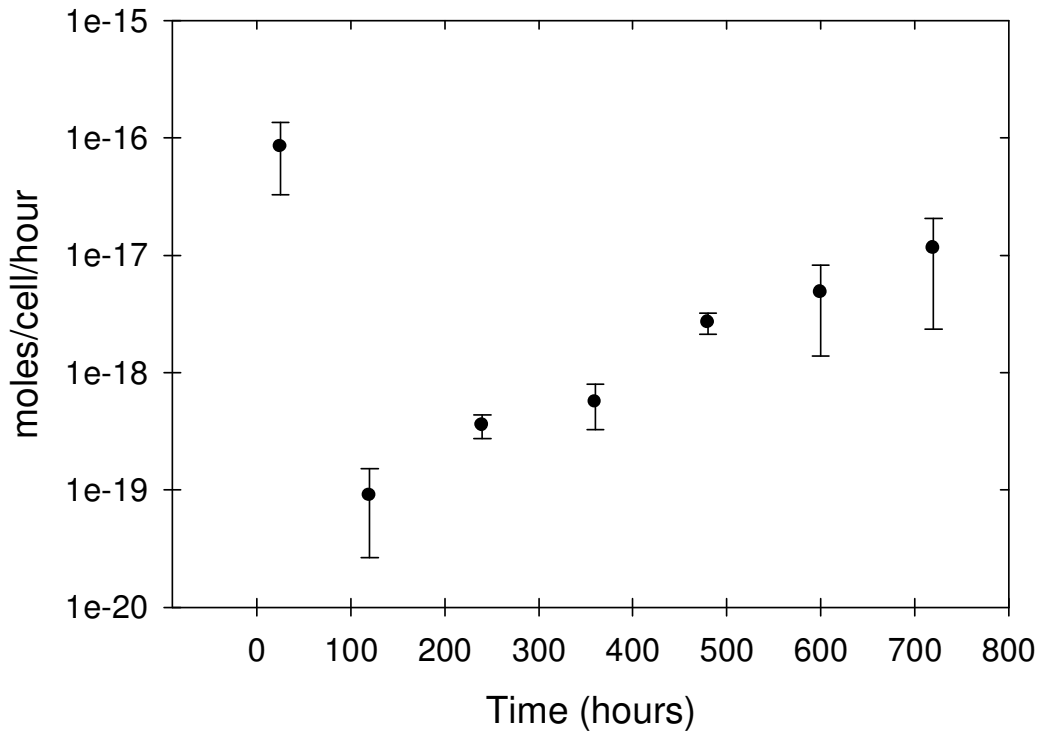

Figure 19

The natural variation within the triplicate samples of dimethyl sulfide (DMS) flux production in moles/cell/ hr over time of the ¼ silica nutrient treatments.

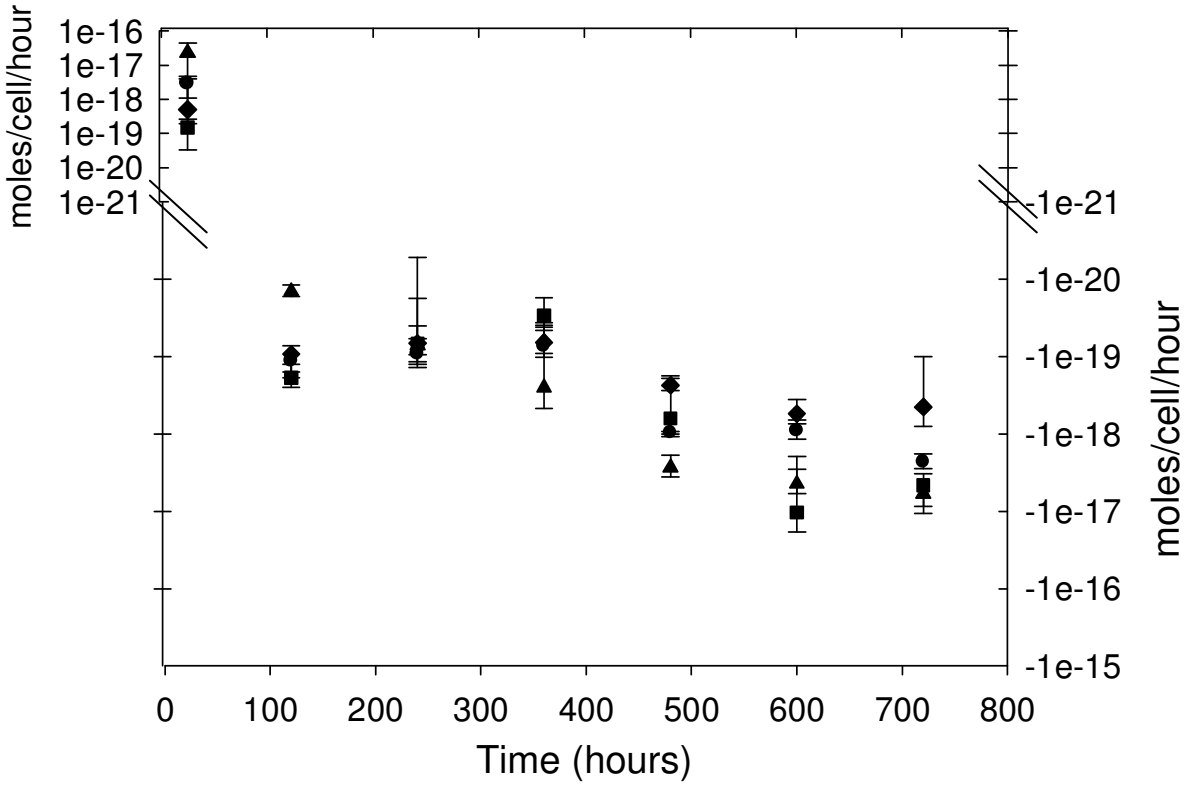

Figure 20

Average fluxes of 2,3-dimethyl pentane (2,3-DMP) produced in mole/cell/hour over the 30 day period. Samples of control (full nutrient treatments) (squares), ½ phosphate nutrient treatments (diamonds), ½ nitrate nutrient treatments (circles), and ¼ silica treatments (triangles). The graph contains two different axes. The one of the left is negative production of 2,3-DMP, the one the right at the top contains the positive production of 2,3-DMP. The graph is shown with dash lines to each side to signify the broken portion which omitted section between the positive and negative values.

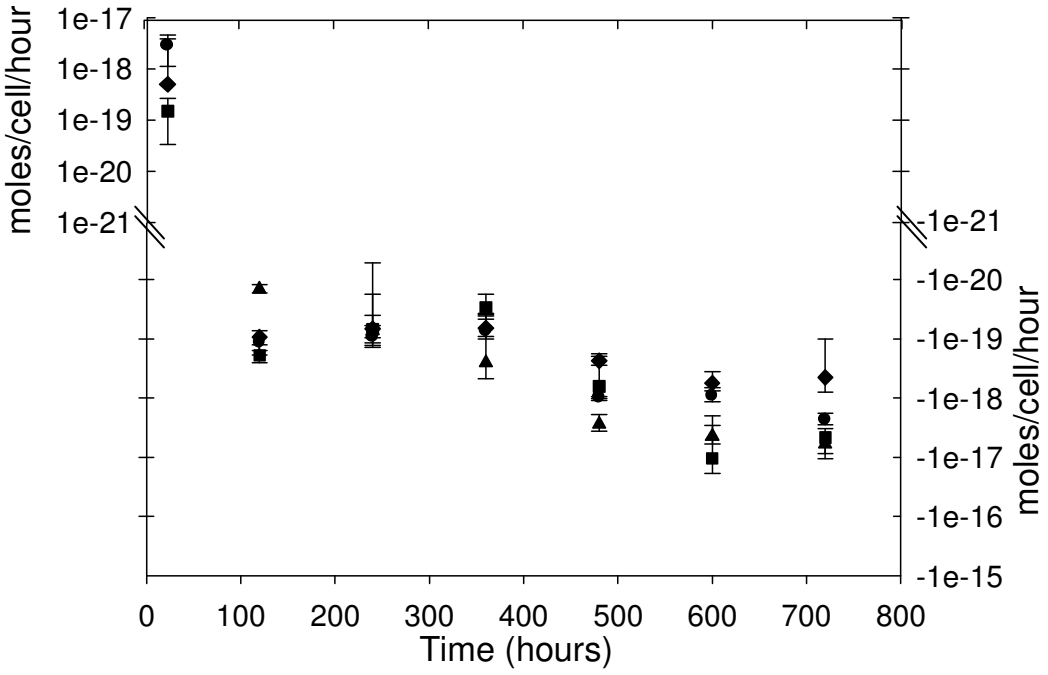

Figure 21

Average fluxes of 2,3-dimethyl pentane (2,3-DMP) produced in mole/cell/hour over the 30 day period with the removal of observation one of silica at 25 hour incubation. Samples of control (full nutrient treatments) (squares), ½ phosphate nutrient treatments (diamonds), ½ nitrate nutrient treatments (circles), and ¼ silica treatments (triangles). The graph contains two different axes. The one of the left is up take of 2,3-DMP, the one the right at the top contains the positive production of 2,3-DMP. The graph is shown with dash lines to each side to signify the broken portion which omitted section between the positive and negative values.

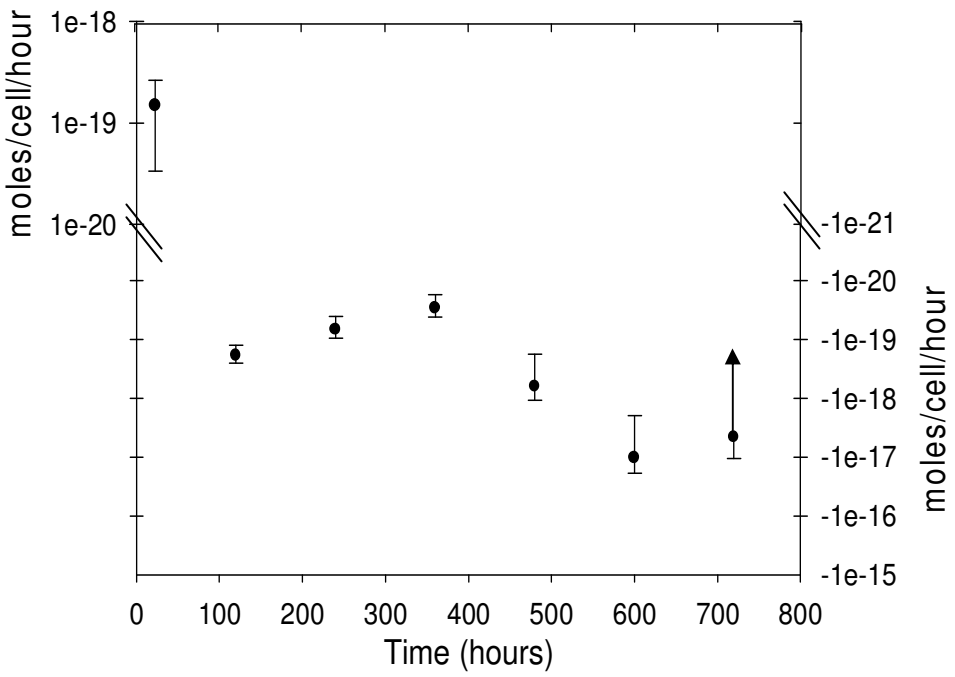

Figure 22

The natural variation within the triplicate samples of 2,3-dimethyl pentane (2,3-DMP) flux production in moles/cell/ hr over time of the control (full nutrient treatments). The graph contains two different axes. The one of the left is negative production of 2,3-DMP, the one the right at the top contains the positive production of 2,3-DMP. The graph is shown with dash lines to each side to signify the broken portion which omitted section between the positive and negative values. Points with arrows contain error bars which extend pass the graph area.

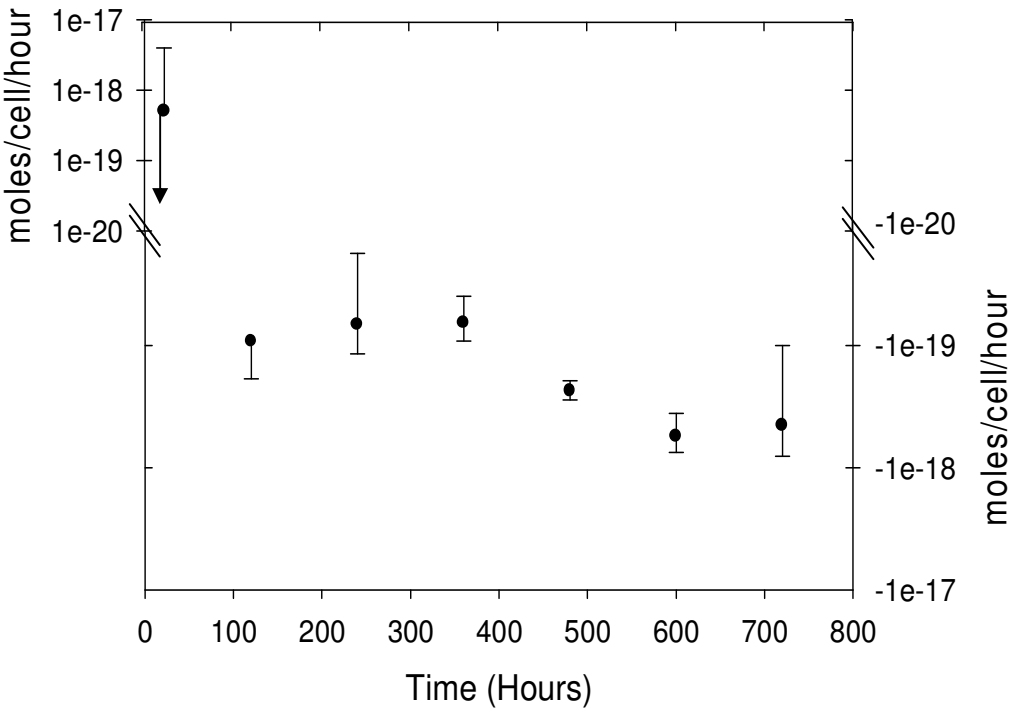

Figure 23

The natural variation within the triplicate samples of 2,3-dimethyl pentane (2,3-DMP) flux production in moles/cell/ hr over time of the ½ phosphate nutrient treatments. The graph contains two different axes. The one of the left is negative production of 2,3-DMP, the one the right at the top contains the positive production of 2,3-DMP. The graph is shown with dash lines to each side to signify the broken portion which omitted section between the positive and negative values. Points with arrows contain error bars which extend pass the graph area.

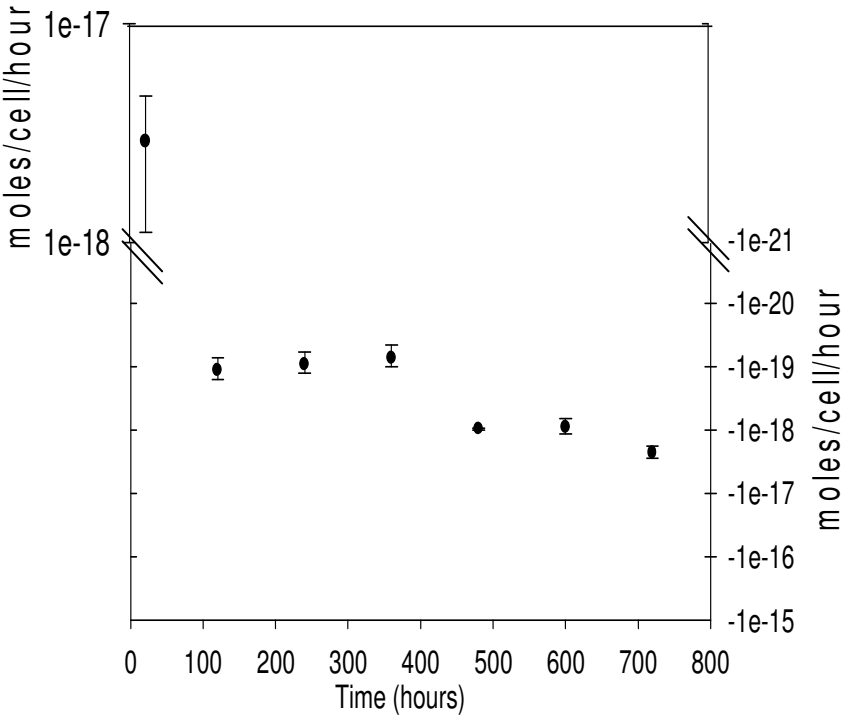

Figure 24

The natural variation within the triplicate samples of 2,3-dimethyl pentane (2,3-DMP) flux production in moles/cell/ hr over time of the ½ nitrate nutrient treatments. The graph contains two different axes. The one of the left is negative production of 2,3-DMP, the one the right at the top contains the positive production of 2,3-DMP. The graph is shown with dash lines to each side to signify the broken portion which omitted section between the positive and negative values.

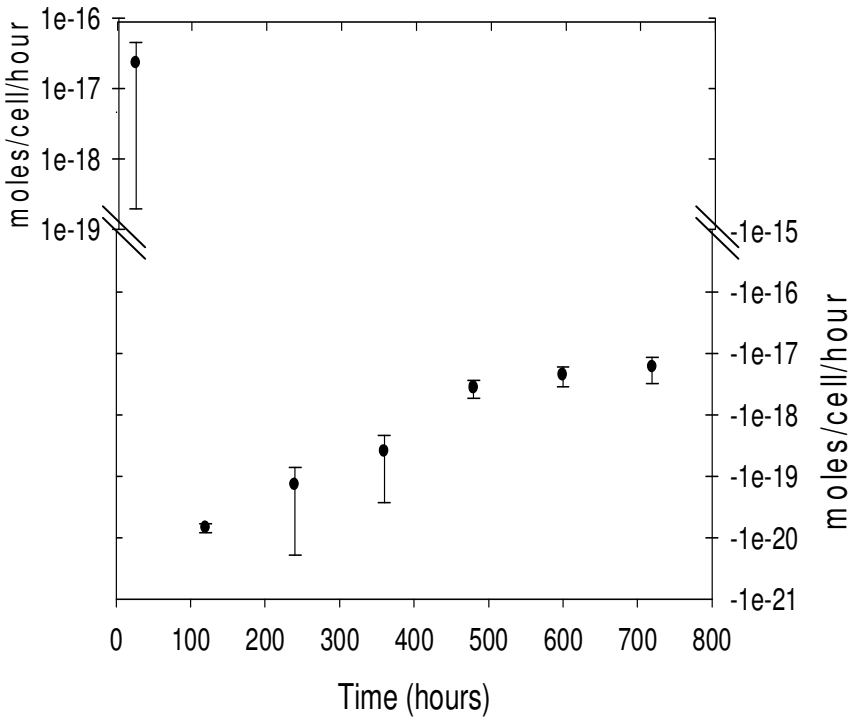

Figure 25

The natural variation within the triplicate samples of 2,3-dimethyl pentane (2,3-DMP) flux production in moles/cell/ hr over time of the ¼ silica nutrient treatments. The graph contains two different axes. The one of the left is negative production of 2,3-DMP, the one the right at the top contains the positive production of 2,3-DMP. The graph is shown with dash lines to each side to signify the broken portion which omitted section between the positive and negative values.

F/2 Components	Individual Compounds	Concentrations in moles	Liter of water dissolved in to	Total added
Nutrients	$Na_2SiO_3 \cdot 9H_20$ $NaH_2PO_4 \cdot H_20$ $NaNO_3$ $H2SeO_3$	.044 .018 .440 .025	0.5 0.5 0.5 1	1 mL per 1 L media
Working Stock of Trace Metals	Na_2EDTA $FeCl_3 \cdot 6H_2O$ Primary Stock Trace Metals	.0056 .0059 Added 0.5 mL	0.5 0.5	0.5 mL to Primary Stock
Primary Stock Trace Metals	$CuSO_4 \cdot H_20$ $ZnSO_4 \cdot 7H_2O$ $CoCl_2 \cdot 6H_2O$ $MnCl_2 \cdot 4H_2O$ $NaMoO_4 \cdot 2H_2O$	.00055 .00077 .00025 .0011 .00029	0.01 0.01 0.01 0.01 0.01	1 mL per L media
Working Stock of Vitamins	Thiamine HCL Biotin B_{12}	7.5 x 10-5 .00041 .00074	0.1 0.1 0.1	0.1 mL to Primary Stock

EDTA: $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$

Table 1

F/2 nutrient treatment components.

Compounds	Retention time (min)	Reference Concentrations in ppb	Sensitivity g/ pA sec
Isoprene	~3.6	212.7365	7.92605E-10
DMS	~4.3	246.621	1.5774E-09
2,3-DMP	~4.5	73.84	1.20548E-09

Table 2

Table includes compounds retention time on column, concentration in reference gas, and overall sensitivity in grams picoamps^{-1}seconds^{-1}

Isoprene Flux Data

	Control		½ Phosphate		½ Nitrate		¼ Silica	
time (hr)	moles/cell/hr	Stand. Dev.	moles/cell/hr	Stand. Dev.	moles/cell/hr	Stand. Dev.	moles/cell/hr	Stand. Dev.
25	1.23E-19	3.29E-20	1.87E-19	0	4.84E-19	2.97E-19	5.26E-18	5.34E-18
120	7.23E-20	8.84E-21	3.69E-20	1.91E-20	5.55E-20	2.20E-20	2.52E-20	2.12E-20
240	1.14E-20	2.73E-21	1.16E-20	3.05E-21	1.99E-20	1.59E-20	5.48E-20	1.58E-21
360	3.30E-21	7.56E-22	5.03E-21	1.98E-21	6.38E-21	2.21E-21	2.52E-20	7.72E-21
480	3.29E-20	9.78E-21	2.55E-21	0	1.51E-19	6.88E-20	6.93E-19	7.60E-19
600	3.94E-19	9.15E-20	8.94E-21	2.54E-21	2.35E-20	1.29E-20	4.12E-19	3.60E-19
720	4.45E-19	4.00E-19	1.51E-20	5.76E-21	6.95E-20	1.11E-20	8.75E-19	1.10E-18

Table 3

Stand. Dev.: Standard deviation of the three individual samples from their average**.**

Table 3 is the average fluxes of isoprene produced in mole/cell/hour listed per sampling period of the control (full nutrient treatments) , ½ phosphate nutrient treatments , 1.2 nitrate nutrient treatments (triangles), and ¼ silica nutrient treatments .

DMS Flux Data

	Control		½ Phosphate		½ Nitrate		¼ Silica	
time (hr)	moles/cell/hr	Stand. Dev.	moles/cell/hr	Stand. Dev.	moles/cell/hr	Stand. Dev.	moles/cell/hr	Stand. Dev.
25	5.47E-19	1.65E-10	2.36E-19	9.53E-11	5.55E-18	4.89E-10	8.40E-17	1.28E-09
120	4.24E-19	5.12E-10	2.49E-19	1.15E-09	4.07E-19	1.23E-09	1.12E-19	2.79E-09
240	1.68E-19	1.20E-09	2.09E-19	2.46E-09	3.48E-19	2.03E-09	4.44E-19	1.87E-09
360	6.14E-20	3.83E-09	1.63E-19	1.86E-09	1.87E-19	2.25E-09	7.01E-19	3.03E-09
480	1.77E-18	4.12E-09	1.49E-19	4.04E-09	1.84E-18	6.60E-10	3.35E-18	1.43E-09
600	2.24E-17	3.88E-09	1.01E-18	3.78E-09	2.10E-18	2.35E-09	6.03E-18	6.69E-09
720	1.44E-17	6.65E-09	1.46E-18	9.01E-09	7.98E-18	1.47E-08	1.44E-17	1.37E-08

Table 4

Stand. Dev.: Standard deviation of the three individual samples from their average**.**

Tabel 4 is the average fluxes of dimethyl sulfide (DMS) produced in mole/cell/hour listed per sampling period of the control (full nutrient treatments) , ½ phosphate nutrient treatments , 1.2 nitrate nutrient treatments, and ¼ silica nutrient treatments.

2,3-DMP Flux Data

	Control		½ Phosphate		½ Nitrate		¼ Silica	
time (hr)	moles/cell/hr	Stand. Dev.	moles/cell/hr	Stand. Dev.	moles/cell/hr	Stand. Dev.	moles/cell/hr	Stand. Dev.
25	1.48E-19	1.15E-19	5.01E-19	3.43E-18	2.89E-18	1.78E-18	2.26E-17	2.24E-17
120	-2.4E-19	7.7E-20	-1.2E-19	1.17E-19	-1.4E-19	5.38E-20	-1.8E-20	3.02E-21
240	-8.5E-20	3.48E-20	-8.4E-20	6.23E-20	-1.2E-19	4.29E-20	-9E-20	8.36E-20
360	-3.7E-20	1.5E-20	-8.2E-20	3.26E-20	-9.3E-20	3.5E-20	-3.2E-19	2.7E-19
480	-7.9E-19	5.71E-19	-2.9E-19	5.21E-20	-1.2E-18	4.22E-20	-3.4E-18	1.09E-18
600	-1.3E-17	1.05E-17	-6.9E-19	2.41E-19	-1.1E-18	3.14E-19	-5.5E-18	1.95E-18
720	-5.8E-18	7.46E-18	-5.6E-19	4.39E-19	-2.9E-18	6.38E-19	-7.5E-18	3.43E-18

Table 5

Stand. Dev.: Standard deviation of the three individual samples from their average

Table 5 is the average fluxes of 2,3-dimethyl pentane (2,3-DMP) produced in mole/cell/hour listed per sampling period of the control (full nutrient treatments) , ½ phosphate nutrient treatments , 1.2 nitrate nutrient treatments, and ¼ silica nutrient treatment.