



NRL/MR/6110--08-9138

Desalination Technology Waste Streams: Impact of pH and Brine on Bacterial Metabolism Among Natural Marine Assemblages

M. T. MONTGOMERY

T. J. BOYD

C. L. OSBURN

R. E. PLUMMER

R. B. COFFIN

*Chemical Dynamics and Diagnostics Branch
Chemistry Division*

September 10, 2008

20081014090

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 10-09-2008		2. REPORT TYPE Memorandum Report		3. DATES COVERED (From - To) 1 January 2005 - 31 December 2008	
4. TITLE AND SUBTITLE Desalination Technology Waste Streams: Impact of pH and Brine on Bacterial Metabolism Among Natural Marine Assemblages				5a. CONTRACT NUMBER 61-5557-M65	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) M. T. Montgomery, T. J. Boyd, C. L. Osburn, R. E. Plummer and R. B. Coffin				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Naval Research Laboratory 4555 Overlook Avenue, SW Washington, DC 20375-5320				8. PERFORMING ORGANIZATION REPORT NUMBER NRL/MR/6110--08-9138	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Strategic Environmental Research and Development Program 901 North Stuart Street, Suite 303 Arlington, VA 22203-1995				10. SPONSOR / MONITOR'S ACRONYM(S) SERDP	
				11. SPONSOR / MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Hydrate formation-based techniques have been proposed as desalination technologies for transforming seawater into potable water. Marine Desalination Systems (MDS) is currently developing new technology in gas hydrate formation to supply potable water using hydrocarbon gas-based hydrate crystals (Kubota et al. 1984). The MDS technology might change the natural bacterial assemblage in primarily two ways: metabolic rate (secondary production) and assemblage composition. This influence on the microbial assemblage can affect ecosystem health with a disruption of the microbial growth efficiency and changes to key elemental cycles. This report focuses on the effect of salinity and pH changes on the rates of bacterial metabolism among natural marine assemblages. When the marine bacterial assemblage is exposed to salinity and pH conditions similar to those expected in MDS system waste streams, heterotrophic production is significantly reduced. However, in the case of salinity, these impacts on overall heterotrophic bacterial metabolism may be transient. Bacterial production inhibition due to decrease in pH is dramatic and appears much less reversible based on the production recovery only after 24 hours. It should be noted that the pH change was three orders of magnitude compared with the two-fold maximum salinity change. The inhibitory effect of 1.5 pH units (from pH 8.0 to 6.5) at 33 PSU was approximately equal to increasing the salinity 27 PSU (from 33 to 60 PSU) at pH 8.0.					
15. SUBJECT TERMS Desalination Salinity Bacteria pH Heterotrophic Hydrates					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UL	18. NUMBER OF PAGES 16	19a. NAME OF RESPONSIBLE PERSON Michael T. Montgomery
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code) (202) 404-6419

CONTENTS

Abstract.....	1
Introduction.....	1
Material and Methods	2
Water sample collection.....	2
Salinity	2
Changes in pH.....	2
Heterotrophic bacterial production	3
Pressure chamber experiments.....	3
Results and Discussion	3
Conclusions.....	4
Acknowledgements.....	5
Disclaimer.....	5
References.....	5
List of Figures	7

DESALINATION TECHNOLOGY WASTE STREAMS: IMPACT OF pH AND BRINE ON BACTERIAL METABOLISM AMONG NATURAL MARINE ASSEMBLAGES

ABSTRACT

Hydrate formation-based techniques have been proposed as desalination technologies for transforming seawater into potable water. Marine Desalination Systems (MDS) is currently developing new technology in gas hydrate formation to supply potable water using hydrocarbon gas-based hydrate crystals (Kubota et al. 1984). The MDS technology might change the natural bacterial assemblage in primarily two ways: metabolic rate (secondary production) and assemblage composition. This influence on the microbial assemblage can affect ecosystem health with a disruption of the microbial growth efficiency and changes key elemental cycles. This report focuses on the effect of salinity and pH changes on the rates of bacterial metabolism among natural marine assemblages. When the marine bacterial assemblage is exposed to salinity and pH conditions similar to those expected in MDS system waste streams, heterotrophic production is significantly reduced. However, in the case of salinity, these impacts on overall heterotrophic bacterial metabolism may be transient. Bacterial production inhibition due to decrease in pH is dramatic and appears much less reversible based on the production recovery only after 24 hours. It should be noted that the pH change was three orders of magnitude compared with the two-fold maximum salinity change. The inhibitory effect of 1.5 pH units (from pH 8.0 to 6.5) at 33 PSU was approximately equal to increasing the salinity 27 PSU (from 33 to 60 PSU) at pH 8.0.

INTRODUCTION

Hydrate formation-based technologies can be used for desalination, transforming seawater into potable water (Miller 2003). Under low temperature and high pressure, hydrocarbon gases dissolved in seawater form clathrate crystals that exclude sea salts and dissolved organic matter (Barduhn 1982). If separated from the brine prior to melting, the clathrate crystals can entrain potable water, easily separated from purgeable hydrocarbon gas (Barduhn 1982). Physical separation of the clathrate ice crystals from the aqueous brine prior to melting has been a technological challenge to implementing this strategy (McCormack and Niblock 2000, Miller 2003). In addition, release of wastewater containing elevated salinity and dissolved organic matter concentration can locally affect natural bacterial assemblages, especially when strategies that form clathrate crystals are deployed in the deep ocean.

Marine Desalination Systems (MDS) is currently developing new technology in gas hydrate formation to supply potable water. To scale this technology to support different country needs, environmental impacts must be assessed. It is important to determine the MDS technology impact on local water quality and marine microbiota through partitioning of dissolved organic carbon and brine production in waste streams. High local fluctuations in salinity that would inevitably result from the hydrate formation may be comparable to those seen in sea ice formation. Understanding changes in the marine microbiota can provide the capability to predict changes in the ecosystem health.

The MDS technology can affect the natural bacterial assemblage in primarily two ways: metabolic rate (secondary production) and assemblage composition. Changing water quality can affect the microbial assemblage by reducing microbial production, which would result from changes in organic substrate concentration or salinity. Previous experiments assessed the effect of the MDS process on water quality and the growth rate of the natural bacterial assemblage (Coffin et al. 2006). Coffin et al. (2006) measured heterotrophic bacterial production, dissolved organic carbon (DOC) concentration and stable isotope values, and fluorescence spectra of the organic matter that partitions into the hydrate and wastewater streams. They found that the MDS process appeared to inhibit bacterial growth based on the difference in production between the source water and process water samples. This report focuses on the effect of salinity and pH changes on the rates of bacterial metabolism of the natural marine assemblage.

MATERIAL AND METHODS

Water sample collection

Samples were collected in 250 mL Nalgene bottles previously acid-washed and rinsed with MilliQ grade water. Three experiments were conducted using coastal surface water samples collected from (1) Delaware Bay (Lewes, DE) on 7 December 2006, (2) Atlantic Ocean (Indian River, DE) on 14 July 2007, and (3) Pacific Ocean (Honolulu, HI) on 13 August 2007. For experiment (1), Delaware Bay surface water was collected from the Lewes-Cape Henlopen pier. For experiment (2), Atlantic Ocean surface seawater was collected from Indian River Inlet, DE on an incoming tide. For experiment (3), Pacific Ocean surface seawater was collected from a pier in Honolulu.

Salinity

To increase salinity, NaCl (17.55 mg mL⁻¹), KCl (1.33 mg mL⁻¹), MgSO₄ (12.38 mg mL⁻¹), and CaCl₂ (1.11 mg mL⁻¹) (Sigma Chemical, St. Louis, MO) were added to subsamples of water. The salts were baked prior to use to remove any organic carbon. Because it was not possible to make a sufficiently concentrated stock solution, appropriate amounts of each salt were added to 200 mL subsamples of surface water for final salinities of 29, 33, 39, 47, 56, and 60 Practical Salinity Units (PSU) for experiment (1); 33, 41, 50, 60, and 68 PSU for experiment (2); and 30, 40, 50, 60, and 70 PSU for experiment (3). Salinity was determined with a refractometer. The highest salinity concentrations examined are typical of desalination effluent (Raventos et al. 2006).

Changes in pH

CO₂ gas was bubbled into the water sample for up to 45 minutes and pH changes were monitored with a pH meter during the sample preparation. The final pH of treatments were 8.0 (no bubbling), 7.55, 6.50, 5.53, and 4.88 for experiment (1). For experiment (2), the salinity was adjusted prior to bubbling with CO₂, so each salinity treatment has pH adjusted separately to 8.03 (no bubbling), 6.60, 4.91 for 33 PSU; 8.05, 6.78, and 4.94 for 41 PSU; 7.99, 6.32, and 4.92

for 50 PSU; 7.98, 6.49, and 4.95 for 60; and, 7.91, 6.63, and 4.97 for 68 PSU. For experiment (3), the pH treatments were 8.0 (no bubbling) and pH 4.9.

Heterotrophic bacterial production

The leucine incorporation method (Kirchman et al. 1985, Kirchman 1993, Smith and Azam 1992) was used to measure bacterial production. A 1.0 mL water sample from each treatment was added to 2 mL centrifuge tubes (three experimental and one control) which were pre-charged with [^3H -4,5]-L-leucine ($154 \text{ mCi mmol}^{-1}$). Samples were incubated for 2 h at *in situ* temperatures and subsequently processed by the method of Smith and Azam (1992) but centrifuging for 7 min instead of 10 min. A constant isotope dilution factor of 1000 was used for all samples. This was estimated from actual measurements of sediment dissolved free amino acids (Burdige and Martens 1990) and saturation experiment estimates (Tuominen 1995). Leucine incorporation rate was converted to bacterial carbon using factors determined by Simon and Azam (1989).

Pressure chamber experiments

Pacific Ocean surface water (100 mL) was incubated for 72 hours at 25°C and *in situ* pressure corresponding to a 600 m depth in a laboratory pressure facility at the University of Hawaii (Coffin et al. 2004). Prior to incubation, 50 mL of sample was bubbled with CO_2 for 45 min to lower the pH from 8.0 to 4.9. The two samples were pressurized in separate incubation chambers. After 72 hours, the chambers were depressurized to ambient laboratory pressure. One mL aliquots were placed in 2 mL microfuge tubes and then salinity was amended with 10 min of depressurization. After one hour of depressurization, one set of tubes representing each pH (4 and 8) and each salinity (30, 40, 50, 60, and 70 PSU) were processed for bacterial production. The second set was processed 24 hours after depressurization.

RESULTS AND DISCUSSION

This desalination process using hydrate formation may influence the growth of the natural bacterial assemblage through several ways including changes in salinity, pH, DOC concentration, DOC quality and pressure. The effects of two of these parameters, pH and salinity, on heterotrophic bacterial production were examined in three experiments involving surface water from the Delaware Bay, Atlantic Ocean and Pacific Ocean.

The rate of heterotrophic bacterial production of surface water taken from the mouth of the Delaware Bay (December 2006) decreased linearly ($R^2 = 0.95$) with increasing salinity from 29.5 to 60 PSU at the ambient pH 8.0 (Figure 1). This represents a 57% decrease in production from $420 (\pm 20) \text{ ng C L}^{-1} \text{ d}^{-1}$ in the ambient salinity (29.5 PSU) to $180 (\pm 10) \text{ ng C L}^{-1} \text{ d}^{-1}$. This is very similar to the 67% decrease with salinity increase from 33 to 68 PSU in the Atlantic Ocean surface water sample (July 2007)(Figure 2) despite the large difference in production of the respective unamended samples (0.42 ± 0.02 versus $17.9 \pm 1.9 \mu\text{g C L}^{-1} \text{ d}^{-1}$). This latter difference is very likely due to seasonal difference in the sample collection times.

The effect of pH was also very similar between these two sampling events. The rate of heterotrophic bacterial production of Delaware Bay surface water decreased 96% when pH was lowered from its initial value of 8.00 to 4.88 with bubbling by CO₂ at 29.5 PSU salinity (Figure 3). Likewise, the bacterial production of Atlantic Ocean surface water decreased close to 100% when pH was lowered from its initial value of 8.00 to 4.99 at salinities ranging from 33 to 68 PSU (Figure 4). In the Atlantic Ocean sample, the combined effects of salinity and pH were examined. Increasing salinity decreased bacterial production at pH 8.0 from 17.9 $\pm$ 1.9 $\mu\text{g C L}^{-1} \text{ d}^{-1}$ at 33 PSU to 5.94 $\pm$ 0.4 $\mu\text{g C L}^{-1} \text{ d}^{-1}$ at 68 PSU. The inhibitory effect of 1.5 pH units (from pH 8.0 to 6.5) at 33 PSU was approximately equal to increasing the salinity 27 PSU (from 33 to 60 PSU) at pH 8.0 (pH 8.0, 60 PSU = 8.02 $\pm$ 0.20 $\mu\text{g C L}^{-1} \text{ d}^{-1}$; pH 6.5, 33 PSU = 6.67 $\pm$ 1.82 $\mu\text{g C L}^{-1} \text{ d}^{-1}$) (Figure 4).

Finally, Pacific Ocean surface water (August 2007) was incubated in a pressure chamber (equivalent to 600 m) for 72 h at 25°C. Prior to incubation, the sample was split into two aliquots with one bubbled with CO₂ for 45 min to reduce the pH to 4.9 while the other was maintained at ambient pH of 8.0. After incubation, both samples were decompressed and salinity of subsamples was adjusted from 30 PSU of the original sample to 40, 50, 60 and 70 PSU. Heterotrophic bacterial production was measured on these samples one-hour post decompression and then 24 hours post decompression. Bacterial production rate ($\mu\text{g C L}^{-1} \text{ d}^{-1}$) decreased 40% with increasing salinity from its initial unamended value of 30 to 70 PSU at pH 8.0 when measured one hour after decompression (Figure 5). However, 24 hours post decompression, bacterial production increased 43% as salinity increased from 30 to 70 PSU. This relationship with salinity was linear for both the one hour (T0) and 24 hour (T1) samplings though the regression was stronger at one hour ($R^2=0.98$) than at 24 hours ($R^2=0.84$) (Figure 5).

The rate of bacterial production ($\mu\text{g C L}^{-1} \text{ d}^{-1}$) showed little relationship with increasing salinity from its initial unamended value of 30 to 70 PSU at pH 4.9 when measured one hour after decompression or 24 hours post decompression (Figure 6). However, bacterial production averaged among all salinities for a given time point increased four-fold from one to 24 hours post decompression (Figure 6). Similarly to that seen with the Delaware Bay and Atlantic Ocean water experiments, decreasing the pH from 8.0 to 4.9 inhibited bacterial production 99.2% even after 24 hours at 33 PSU 20.22 $\pm$ 3.8 versus 0.159 $\pm$ 0.004 $\mu\text{g C L}^{-1} \text{ d}^{-1}$ (Figure 5, 6).

CONCLUSIONS

Increasing salinity causes a decrease in bacterial production in different water samples measured soon after this osmotic change (within one hour). In Pacific Ocean water decompressed for one hour, production measured on a 70-PSU sample decreased 40% relative to the 30-PSU sample. However, 24 hours after decompression, this trend was reversed and production of the 70 PSU sample with 43% higher than the 30-PSU sample. One explanation is that the assemblage that remains active in the elevated salinity environment can increase its metabolism and competition for nutrients. Another possibility is that protozoan grazers may have been disproportionately lysed in the elevated salinity treatments, allowing the bacterial population of slower growing cells to increase in abundance (and likewise increase leucine

incorporation rate at the population level). Bacterial production is significantly reduced exposed to increased salinity and decreased pH conditions similar to those expected in MDS system waste streams. However, in the case of salinity, these impacts on overall heterotrophic bacterial metabolism may be transient (24 hours).

Inhibition of bacterial production due to decrease in pH is dramatic and appears much less reversible based on the recovery of production after 24 hours in experiment (3), though it should be noted that the pH change was three orders of magnitude compared with the two-fold maximum salinity change. The inhibitory effect of 1.5 pH units (from pH 8.0 to 6.5) at 33 PSU was approximately equal to increasing the salinity 27 PSU (from 33 to 60 PSU) at pH 8.0. This impact still needs to be considered for the long-term exposure in the microbial consortium where bacterial production did not vary with pH in deep ocean vents on Loihi, off the coast of Hawaii (Coffin et al., 2004). The reduced pH may alter the microbial assemblage composition, which may affect environmentally important elemental cycles.

ACKNOWLEDGEMENTS

The authors thank Rebecca Plummer at NRL and Charles Nelson at UH for technical support. This work was supported by the Office of Naval Research.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability of responsibility for the accuracy, completeness, or usefulness of any information, apparatus product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

REFERENCES

- Barduhn, A.J. 1982. Desalination by freezing processes, *In*, Encyclopedia of Chemical Processing and Design, McKetta and Cunningham, eds., Marcel Dekker, New York.
- Burdige, D.J., and C.S. Martens. 1990. Biogeochemical cycling in an organic-rich marine basin – 11. The sedimentary cycling of dissolved free amino acids. *Geochim. Cosmochim. Acta.* 54: 3033-3052.

- Coffin, R.B., M.T. Montgomery, T.J. Boyd, and S.M. Masutani. 2004. Influence of ocean CO₂ sequestration on bacterial production. *Energy* 29:1511-1520.
- Coffin, R.B., C.L. Osburn, and M.T. Montgomery. 2006. Impact of a hydrate-based marine desalination technology on marine microbiota and water quality. US Naval Research Laboratory Technical Memorandum, NRL/MR/6110—06-9005.
- Kirchman, D.L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In P.F. Kemp, B. F. Sherr, E.B. Sherr and J.J. Cole. *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers, Washington D.C. pp.509-512.
- Kirchman, D., E. K'nees, and R. Hodson. 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Appl. Environ. Microbiol.* 49: 599-607.
- Kubota, H., K. Shimizu, Y. Tanaka, and T. Makita. 1984. Thermodynamic Properties of R13 (CCIF₃), R23 (CHF₃), R152a (C₂H₄F₂) and Propane Hydrates for Desalination of Sea Water. *J. Chem. Eng. Japan* 17(4);423-429.
- McCormack, R.A., and G.A. Niblock. 2000. Investigation of high freezing temperature, zero ozone, and zero global warming potential clathrate formers for desalination, U.S. Bureau of Reclamation Water Treatment Technology Program Report No. 59, June.
- Miller, J.E. 2003. Review of Water Resources and Desalination Technologies. National Nuclear Security Administration under Contract DE-AC04-94-AL85000, Sandia National Laboratories, Albuquerque, New Mexico, SAND REPORT, SAND 2003-0800.
- Raventos, N., E. Macpherson, and A. Garcia-Rubies. 2006. Effect of brine discharge from a desalination plant on macrobenthic communities in the NW Mediterranean. *Mar. Environ. Res.* 62:1-14.
- Simon, M., and F. Azam. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. *Mar. Ecol. Prog. Ser.* 51:201-213.
- Smith, D.C., and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using ³H-leucine. *Mar. Microb. Food Webs* 6(2):107-114.
- Tuominen, L. 1995. Comparison of leucine uptake methods and a thymidine incorporation method for measuring bacterial activity in sediment. *J. Microbiol. Methods*, 24:125-134.

LIST OF FIGURES

Figure 1. Rate of heterotrophic bacterial production ($\text{ng C L}^{-1} \text{ d}^{-1}$) of surface water taken from the mouth of the Delaware Bay (7 December 2006) decreased 57% with increasing salinity from its initial unamended value of 29.5 to 60 PSU at pH 8.0.

Figure 2. Rate of heterotrophic bacterial production ($\mu\text{g C L}^{-1} \text{ d}^{-1}$) of Atlantic Ocean surface water (14 July 2007) decreased 67% with increasing salinity from its initial unamended value of 33 to 68. Note change in units from ng to μg .

Figure 3. Rate of heterotrophic bacterial production ($\text{ng C L}^{-1} \text{ d}^{-1}$) of surface water taken from the mouth of the Delaware Bay (7 December 2006) decreased 96% when pH was lowered from its initial value of 8.00 to 4.88 with bubbling by CO_2 at 29.5 PSU salinity.

Figure 4. Rate of heterotrophic bacterial production ($\mu\text{g C L}^{-1} \text{ d}^{-1}$) of Atlantic Ocean surface water (14 July 2007) decreased 100% when pH was lowered from its initial value of 8.00 to 4.99 with bubbling by CO_2 at salinities ranging from 33 to 68 PSU.

Figure 5. Pacific Ocean surface water (13 August 2007) was incubated in a pressure chamber (equivalent to 600 m) for 72 h at 25°C . Rate of heterotrophic bacterial production ($\mu\text{g C L}^{-1} \text{ d}^{-1}$) decreased 40% with increasing salinity from its initial unamended value of 30 to 70 PSU at pH 8.0 when measured one hour after decompression (blue diamonds). However, 24 hours post decompression, bacterial production increased 43% as salinity increased from 30 to 70 PSU (black squares).

Figure 6. Pacific Ocean surface water (13 August 2007) was incubated in a pressure chamber (equivalent to 600 m) for 72 h at 25°C . Rate of heterotrophic bacterial production ($\mu\text{g C L}^{-1} \text{ d}^{-1}$) showed little relationship with increasing salinity from its initial unamended value of 30 to 70 PSU at pH 4.9 when measured either one hour after decompression (blue diamonds) or 24 hours post decompression (black squares). However, bacterial production averaged among all salinities for a given time point increased four-fold from one to 24 hours post decompression.

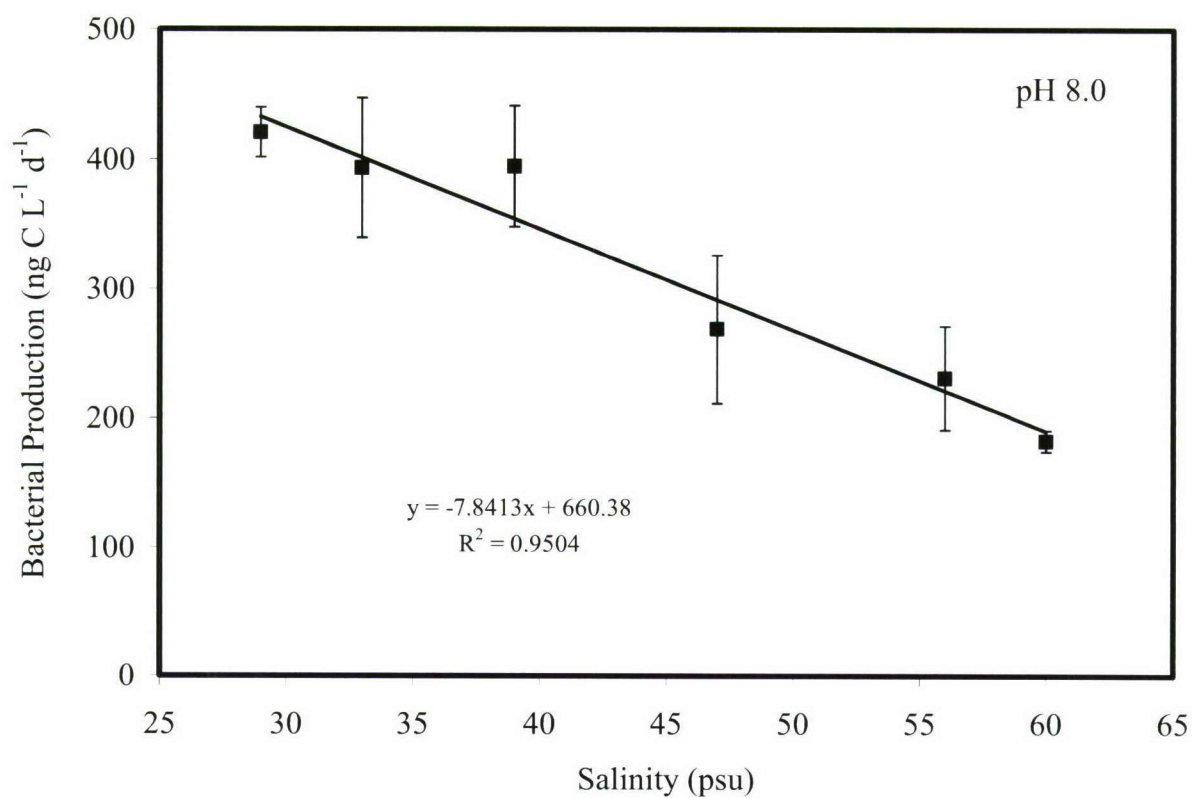


Figure 1. Rate of heterotrophic bacterial production (ng C L⁻¹ d⁻¹) of surface water taken from the mouth of the Delaware Bay (7 December 2006) decreased 57% with increasing salinity from its initial unamended value of 29.5 to 60 PSU at pH 8.0.

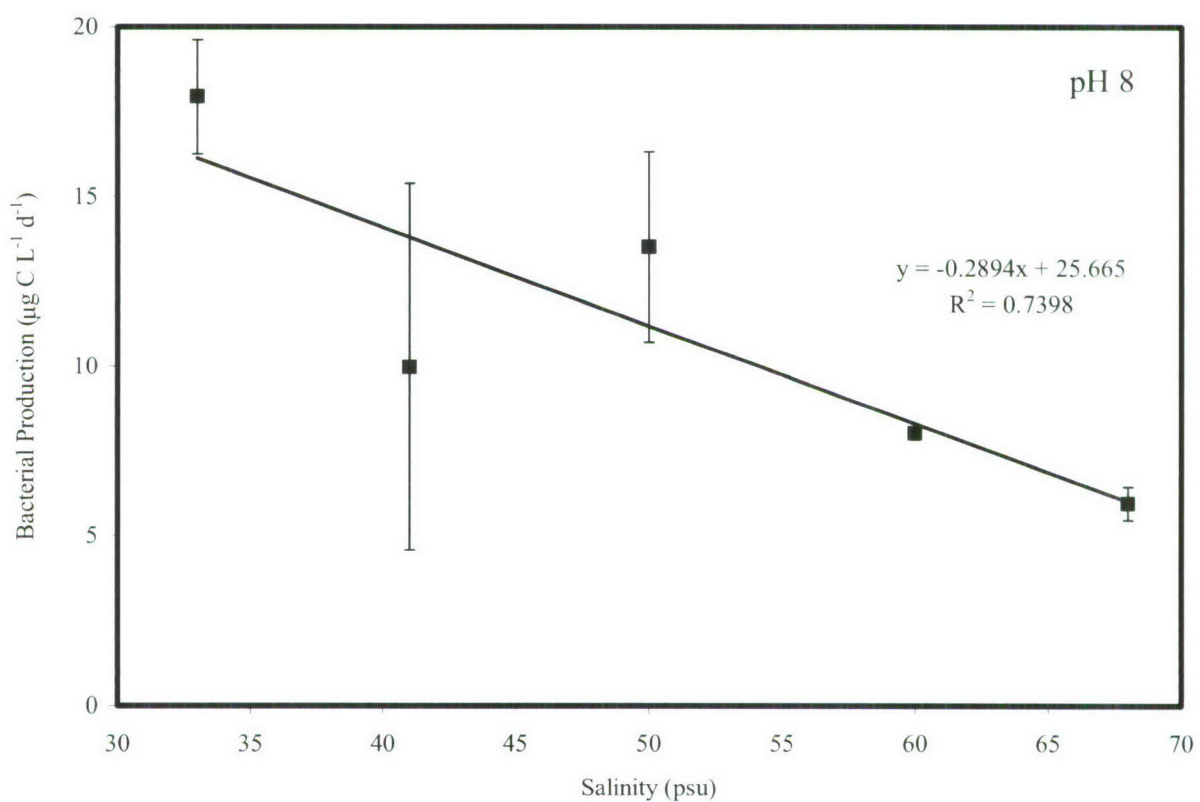


Figure 2. Rate of heterotrophic bacterial production ($\mu\text{g C L}^{-1} \text{d}^{-1}$) of Atlantic Ocean surface water (14 July 2007) decreased 67% with increasing salinity from its initial unamended value of 33 to 68 PSU at pH 8.0. Note change in units from ng to μg .

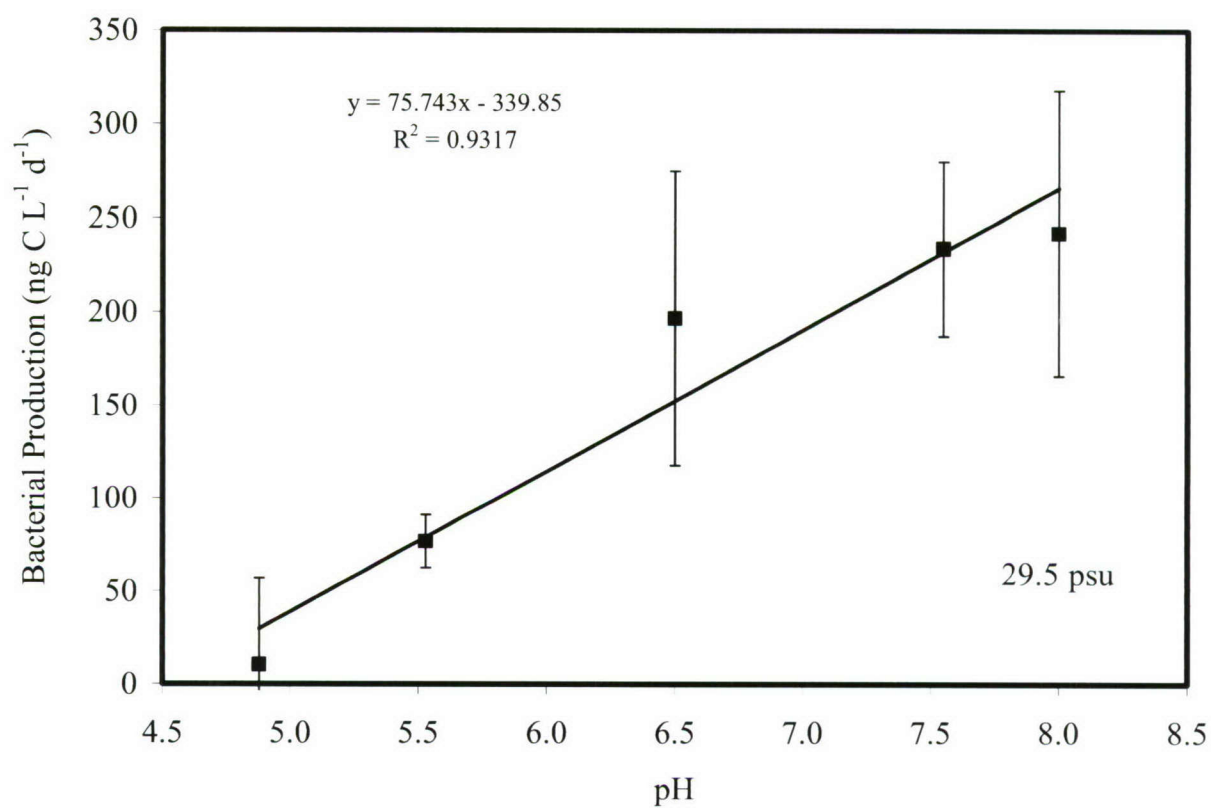


Figure 3. Rate of heterotrophic bacterial production (ng C L⁻¹ d⁻¹) of surface water taken from the mouth of the Delaware Bay (7 December 2006) decreased 96% when pH was lowered from its initial value of 8.00 to 4.88 with bubbling by CO₂ at 29.5 PSU salinity.

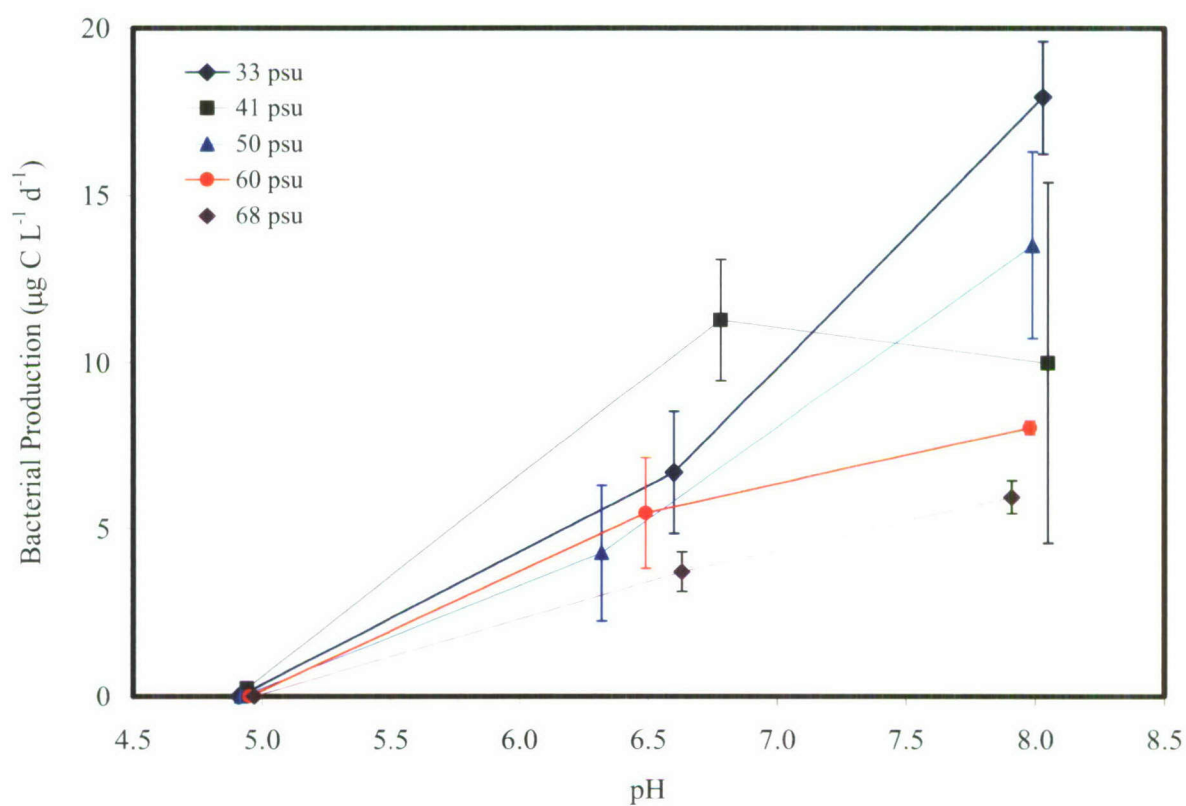


Figure 4. Rate of heterotrophic bacterial production ($\mu\text{g C L}^{-1} \text{d}^{-1}$) of Atlantic Ocean surface water (14 July 2007) decreased 100% when pH was lowered from its initial value of 8.00 to 4.99 with bubbling by CO_2 at salinities ranging from 33 to 68 PSU.

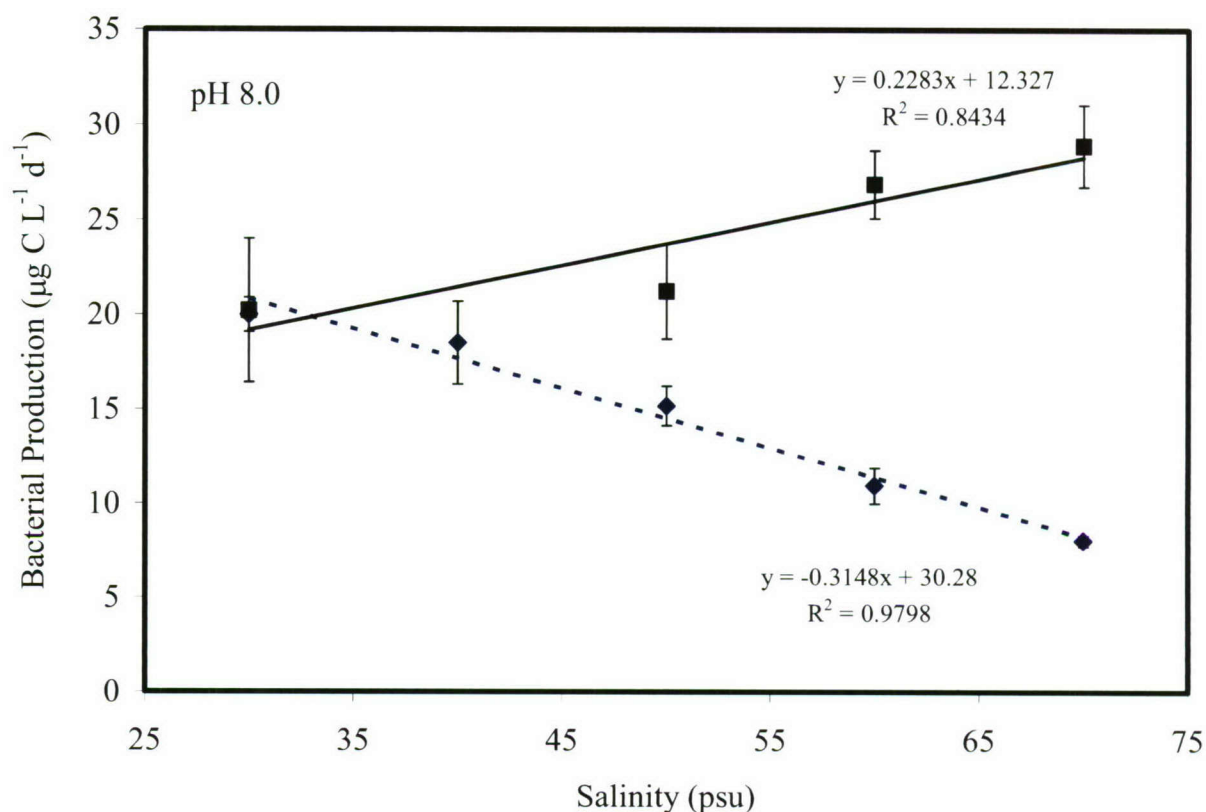


Figure 5. Pacific Ocean surface water (13 August 2007) was incubated in a pressure chamber (equivalent to 600 m) for 72 h at 25°C. Rate of heterotrophic bacterial production ($\mu\text{g C L}^{-1} \text{d}^{-1}$) decreased 40% with increasing salinity from its initial unamended value of 30 to 70 PSU at pH 8.0 when measured one hour after decompression (blue diamonds). However, 24 hours post decompression, bacterial production increased 43% as salinity increased from 30 to 70 PSU (black squares).

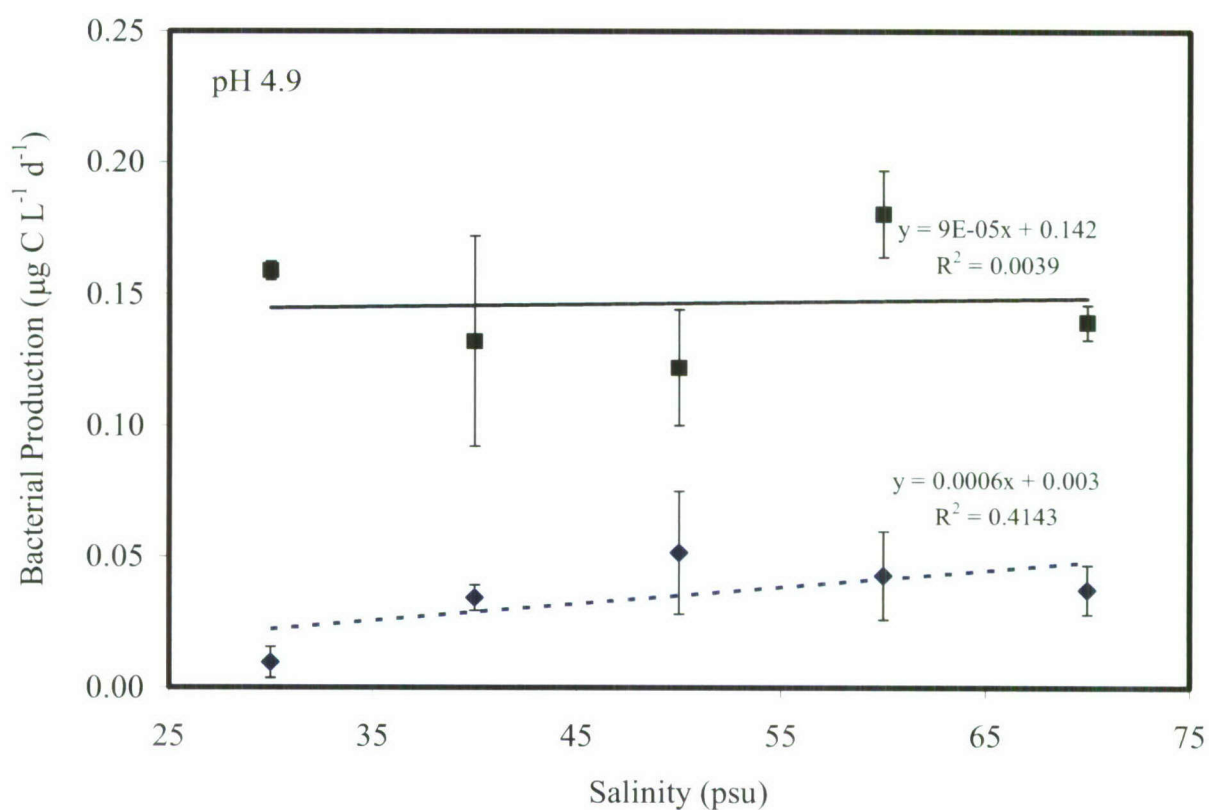


Figure 6. Pacific Ocean surface water (13 August 2007) was incubated in a pressure chamber (equivalent to 600 m) for 72 h at 25°C. Rate of heterotrophic bacterial production ($\mu\text{g C L}^{-1} \text{d}^{-1}$) showed little relationship with increasing salinity from its initial unamended value of 30 to 70 PSU at pH 4.9 when measured either one hour after decompression (blue diamonds) or 24 hours post decompression (black squares). However, bacterial production averaged among all salinities for a given time point increased four-fold from one to 24 hours post decompression.