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OPTIMIZATION OF 15 PARAMETERS INFLUENCING THE LONG-TERM SURVIVAL OF BACTERIA IN AQUATIC SYSTEMS

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NASA is presently engaged in the design and development of a water reclamation system for the future space station. A major concern in processing water is the control of microbial contamination. As a means of developing an optimal microbial control strategy, studies were undertaken to determine the type and amount of contamination which could be expected in these systems under a variety of changing environmental conditions. A laboratory-based Taguchi optimization experiment was conducted to determine the ideal settings for 15 parameters which influence the survival of six bacterial species in aquatic systems. The experiment demonstrated that the bacterial survival period could be decreased significantly by optimizing environmental conditions.

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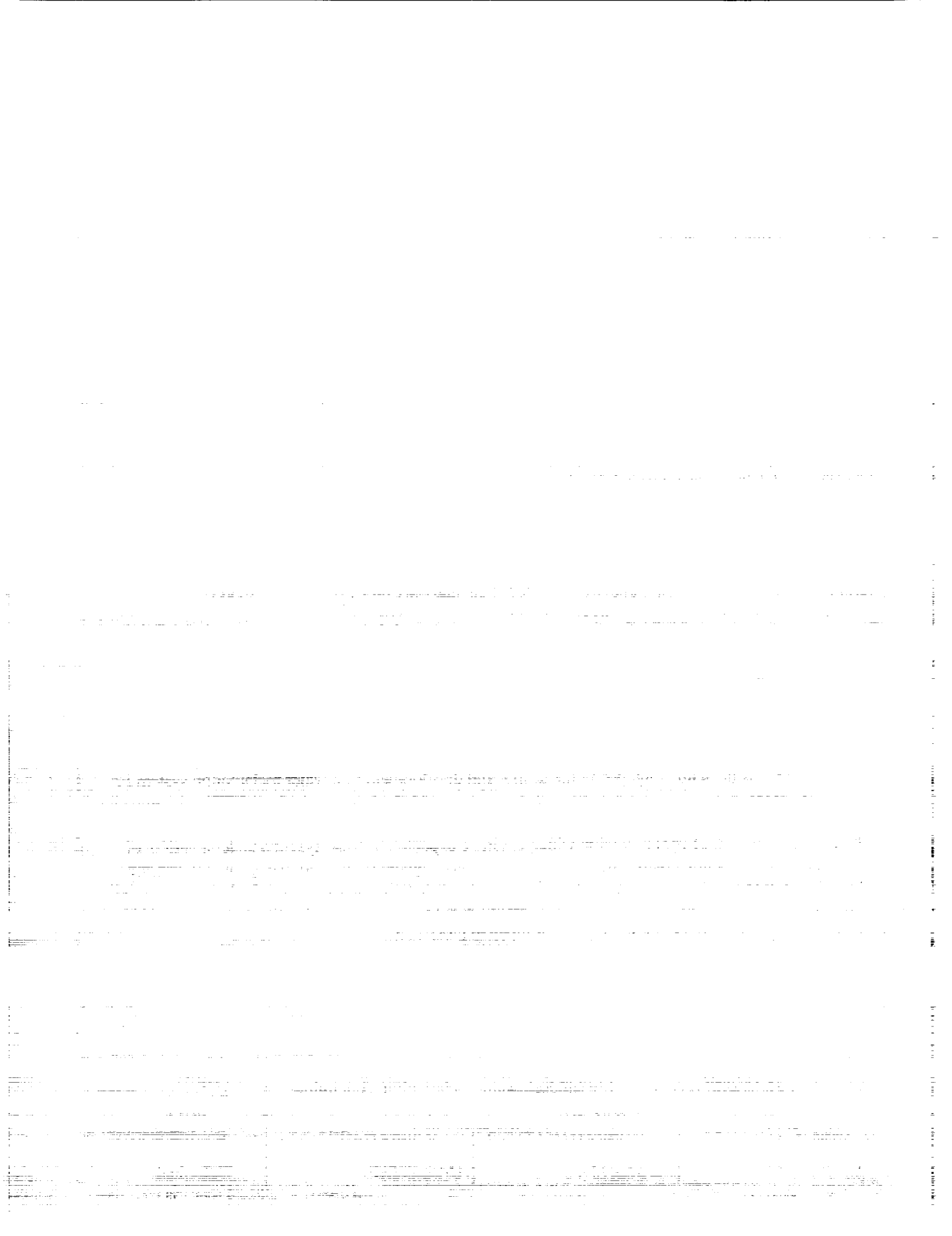


TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Optimization of 15 Parameters Influencing the Long Term Survival of Bacteria in Aquatic Systems

D.C. Obenhuber

BACKGROUND:

NASA is presently engaged in the design and development of an Environmental Control and Life Support System (ECLSS) for the future space station (1). Several aspects of this project depend on systems that handle water as a primary component. These include a water system which will provide potable water for the crew, as well as reclaim waste water (2), a temperature and humidity control system which uses water as a primary coolant, and an ultra pure water facility designed to provide laboratory grade water for experimental purposes. A major concern in handling water in each of these systems is the control of microbial contamination (3,4). Uncontrolled contamination can create a significant health risk and has been implicated in premature system failure as a result of biofouling and microbially influenced corrosion.

As a means of developing an optimal microbial control strategy, studies were undertaken to determine the type and amount of contamination which could be expected in these systems. A variety of different environmental conditions could be expected in each subunit of these water handling systems. The conditions may vary by several parameters based on the source of initial water and treatment processes involved. Some of the most significant variations which could be expected include organic content, pH, redox, temperature, as well as the source and amount of microbial contamination.

Information is available on the survival of many bacterial species under a variety of environmental conditions (5-7). Many of these studies offer insight into the effects of different natural environmental conditions but usually detail the effects of a single parameter on the survival of a particular microorganism. Details on the effects of each parameter in conjunction with all the other parameters are rarely available.

Much of the information on individual parameters has been used in the planning of this experiment which sought to test the effect of 15 parameters on the survival of six species of bacterium. The goal was to derive information which would establish optimum settings for all significant parameters within the limits of the specifications set for normal ECLSS system operation. With this information, the ECLSS environment could be optimized to support no more than the minimal amount of microbial contamination prior to employing disinfection methods. Also, it might be possible to determine the optimal environmental conditions for maximum disinfectant effectiveness.

In seeking the environmental conditions which minimize microbial survival in each particular water system, careful attention must also be given to those conditions which optimize microbial survival. These conditions must be avoided if at all possible. In order to better understand the mechanisms which impart long- term persistence to bacteria, optimal survival conditions must be explored in the laboratory. These mechanisms are currently being studied in a variety of research projects in the microbial ecology facility at NASA's Marshall Space Flight Center. These mechanisms, if better understood, may lead to improved methods of microbial control.

OBJECTIVE:

The objectives of this study were, first, to establish the survival rate (number of surviving bacteria vs. time) for a variety of bacteria commonly found to contaminate aquatic systems. Some of these organisms are natural aquatic inhabitants and others represent genera which include human pathogens. The second objective was to determine the conditions which are most important in the survival of these species. The third was to determine the conditions which would produce minimum microbial survival and, conversely, to determine a set of conditions which would produce maximum microbial survival.

APPROACH:

An optimization experiment was conducted to determine the ideal settings for 15 parameters which influence the survival of six bacterial species in aquatic systems. The use of a traditional two dimensional experimental design which tests the effects of each parameter independently is both time consuming and laborious. An improved experimental design was implemented which utilized a Taguchi two-level fractional factorial (10) to greatly increase the number of possible parameters without significantly increasing the number of experiments. This method permitted the testing of 15 parameters simultaneously in a total of 16 experimental runs as compared to traditional two dimensional design which would have required far more experimental runs (2^15).

The primary drawback of this method is the potential for misleading information as a result of unanticipated interactions between parameters. This study was designed to overcome this problem by using the Taguchi design to eliminate the parameters which have the least influence on microbial survival in aquatic environments and thereby allow analysis of only the significant effects.

METHOD:

In initial studies, the long term survival of six bacterial species was tested in order to select those bacteria which represent the widest variation in aquatic survival period. These bacteria were grown in 10% Brain Heart Infusion (BHI) at 25C for 48 hr (Table 1). The culture density was adjusted to approximately 1.0 E+08 colony forming units per ml (CFU/ml). A 0.1 ml sample of each culture was added to three replicate acid washed test tubes, each containing 10ml of sterile distilled deionized 18 Mohm water (ddH2O) and sealed with a Teflon lined cap. The cultures were stored at room temperature (20-25 C) for 15 months. Microbial survival was assayed by serial dilution and spread plating on R2A incubated at 28 C for seven days. In addition, 0.1 ml samples were added to 10 ml of 10% BHI to test the viability of the bacteria which could not be recovered by plating. Scanning Electron Microscopy (SEM) was used to compare the size and morphology of starved vs. fresh cells. Microbial identification was performed on the Biolog Identification System. Reliability of identification was based on relative comparison with an internal database.

Table 1

Bacteria Studied

Code	Name	ATCC#
PC	*Pseudomonas cepacia*	35254
SA	*Staphylococcus aureus*	6568
SF	*Streptococcus faecalis*	6569
EC	*Escherichia coli*	5922
ST	*Salmonella typhimurium*	14028
SS	*Shigella sonnei*	25931

Following initial studies of bacterial survival, the six bacterial species were used for further testing of 15 parameters which could influence survival. Table 2 lists the 15 parameters tested and the two levels used for each parameter. The choice of parameters was based on the following considerations. Three primary influences were assumed to have significant effects on microbial survival. First, initial growth conditions may affect survival by influencing the metabolic state of bacteria prior to exposure to the stress of a new aquatic environment. Also, initial growth conditions may affect the amount of stored energy reserves of the cell and the regulatory systems currently operating. Second, the physical and chemical conditions of the aquatic environment could have a significant effect on microbial survival. Finally, recovery conditions, though not directly related to the survival process, will significantly affect the data collected and the conclusions drawn.

Each parameter was tested at two levels (Lo and Hi). The conditions selected and the ranges chosen were estimated to be tolerable by the majority of aquatic bacteria, while extreme enough to provide significant variation in response between species. Table 3 displays the design of the experiment by indicating the parameter setting for each run.

The three primary effects of initial growth, environmental conditions and recovery conditions were each subdivided into parameters. For initial growth conditions, the medium composition consisted of either Minimal Broth supplemented with 1% yeast extract (MIN+YE) or Brain Heart Infusion (BHI) broth. The concentration of each of the growth media was prepared at either 10% or 100%. Incubation temperature was set at either 25C or 35C, and cultures were allowed to incubate for either 2 or 11 days.

Table 2

Parameter Selection and Settings

		INITIAL GROWTH CONDITIONS	Lo	Hi
1	GM	Nutrient medium	Minimal	BHI
2	GC	Nutrient concentration (%)	10	100
3	GT	Incubation temperature (C)	25	35
4	GD	Time of incubation (days)	2	11
		ENVIRONMENTAL CONDITIONS		
5	SM	Nutrient medium	Dextrose	BHI
6	SC	Nutrient concentration (mg/l)	0	10
7	SS	Salinity (%NaCl)	0	0.45
8	SP	Phosphate (pH)	5.5	7.2
9	SI	Initial density (CFU/ml)	1.0E+4	1.0E+06
10	ST	Incubation temperature (C)	25	35
11	SO	Oxygen content	anaerobic	aerobic
		RECOVERY CONDITIONS		
12	RM	Nutrient medium	R2A	BHI
13	RC	Nutrient concentration (%)	10	100
14	RT	Incubation temperature (C)	28	35
15	RO	Oxygen content	anaerobic	aerobic

Environmental conditions were similarly varied. Nutrient concentration of the aquatic environment was considered to be zero in deionized distilled water (ddH20) with a conductivity of greater than 18MOhm and a total organic carbon content (TOC) of

less than 20 ug/L. A nutrient concentration of 10mg/L was used in the nutrient supplemented systems. These nutrients include either a low concentration of dextrose, a simple carbon-energy source or BHI, a complex nutrient. Salinity was varied between 0 and 0.45% NaCl. Similarly, the effect of phosphate buffering was controlled by supplementing the media with a standard concentration of Butterfield buffer (pH 7.2). Initial inoculum density was either 1.0E+4 or 1.0E+6 and was produced by dilution in ddH2O of the initial culture. Oxygen concentration was regulated in an anaerobic hood by equilibrating the anaerobic tubes for 24 hours prior to closure. Inoculated tubes were then incubated at either 25C or 35C for four months.

Table 3

Taguchi 16 Run Design (15 factors - 2 levels)
Resolutions 3
(screening, no interactions)

	Growth				Aquatic Environment						Recovery				
Run	GM	GC	GT	GD	SM	SC	SS	SP	SI	ST	SO	RM	RC	RT	RO
1	1	10	25	2	1	0	1	5	4	25	1	1	100	28	1
2	1	10	25	2	1	0	1	7	6	35	2	2	10	35	2
3	1	10	25	11	2	10	2	5	4	25	1	2	10	35	2
4	1	10	25	11	2	10	2	7	6	35	2	1	100	28	1
5	1	100	35	2	1	10	2	5	4	35	2	1	100	35	2
6	1	100	35	2	1	10	2	7	6	25	1	2	10	28	1
7	1	100	35	11	2	0	1	5	4	35	2	2	10	28	1
8	1	100	35	11	2	0	1	7	6	25	1	1	100	35	2
9	2	10	35	2	2	0	2	5	6	25	2	1	10	28	2
10	2	10	35	2	2	0	2	7	4	35	1	2	100	35	1
11	2	10	35	11	1	10	1	5	6	25	2	2	100	35	1
12	2	10	35	11	1	10	1	7	4	35	1	1	10	28	2
13	2	100	25	2	2	10	1	5	6	35	1	1	10	35	1
14	2	100	25	2	2	10	1	7	4	25	2	2	100	28	2
15	2	100	25	11	1	0	2	5	6	35	1	2	100	28	2
16	2	100	25	11	1	0	2	7	4	25	2	1	10	35	1

* non-numeric parameters are represented by 1 at Lo and 2 at Hi settings

Recovery methods used serial dilutions in ddH2O to reduce the concentration of viable cells followed by standard spread plate on the appropriate medium. R2A or BHI agar were used at either full strength or diluted with ddH2O 1 to 10 and supplemented with Bacto Agar to a final agar concentration of 1.5 percent. Plates were then incubated at either 25C or 35C in the

anaerobic hood or room air for seven days prior to counting. Survival of each species was assayed after four months of incubation.

After data analysis, a validation test was conducted to demonstrate that the settings predicted to be optimum for survival produced the maximum or minimum number of surviving cells. In the validation test, each surviving bacterial species was subjected to the set of conditions which had resulted in maximum or minimum survival during the optimization test. The validation test was done periodically to determine the rate of survival and whether survival rate stabilized over time. The settings chosen were based on the highest number of survivors for each parameter and were not necessarily any set of conditions used together during the initial 16 runs.

RESULTS:

In the initial study, all bacterial species tested for long-term aquatic survival in ddH2O were recovered at concentrations of less than 1.0 to greater than 1.0 E+06 CFU/ml after 15 months (Table 4). *P. cepacia* showed a slight increase in numbers from the initial inoculum density. Surviving gram negative isolates were identified and verified by the Biolog Microbial Identification System, except for *Shigella sonnei*. The identification of this species was not sufficient to exceed the minimum acceptable limit of reliability. The six bacterial species were sufficiently different in survival period so that all were required for further study. The surviving cells demonstrated no significant reduction in the rate of growth and could be recovered on R2A in 24-48 hr. Scanning electron microscopic analysis of bacterial cells demonstrated an apparent size reduction of *E. coli* and *P. cepacia* cells from approximately 1.0 um for fresh cultures to approximately 0.3-0.4 um after 15 months. *S. aureus* showed no detectable change in size.

Table 4

Bacterial Survival after 15 Months in Distilled Deionized Water

Bacteria	CFU/ml	Biolog ID	
PC	6.25	Excel	(82%)
SA	<1	none	
SF	<1	none	
EC	4.57	Excel	(87%)
ST	2.87	Good	(76%)
SS	2.74	Poor	(19%)

<1 negative plate count but positive broth culture

Results for standard plate counts of recoverable bacteria from the 15 parameter Taguchi survival experiment are listed in Table 5. It can be seen from the raw data that, of the 16 runs, there are obvious differences in the number of survivors for all but *S. faecalis* which did not survive any of the treatments. Run 14 produced the maximum number of survivors for all bacteria tested except *E. coli*. This observation suggests that the settings for run 14 were optimal, but because all possible settings were not tested in this part of the experiment, further information on optimal survival had to be derived from individual parameter analyses.

Table 5

Microbial Survival of Taguchi Designed Experiment

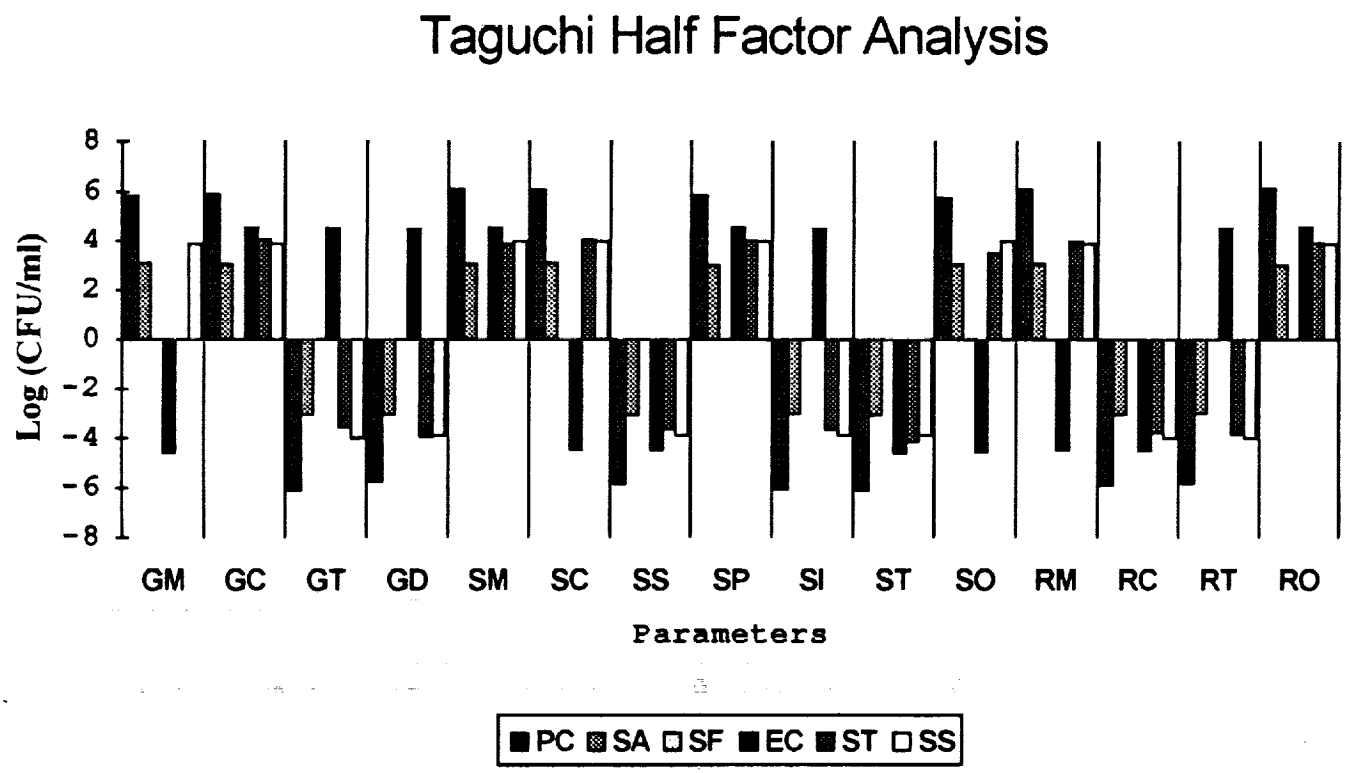
Data Summary
Log (CFU/ml)

run	PC	SA	SF	EC	ST	SS
1	0	0	0	0	0	0
2	4.4	0	0	0	0	0
3	6.7	2.5	0	4.6	4.7	0
4	0	0	0	0	0	4.2
5	4.6	0	0	3.9	4.0	0
6	0	0	0	4.5	4.9	0
7	0	0	0	0	0	0
8	6.0	0	0	5.6	4.5	0
9	4.4	1.4	0	3.7	3.8	3.0
10	0	0	0	2.7	4.6	2.8
11	0	2.9	0	0	3.6	0
12	5.3	0	0	0	0	0
13	0	2.9	0	2.4	3.5	0.5
14	7.2	4.3	0	4.4	5.1	5.1
15	5.9	0	0	3.0	3.1	0
16	0	0	0	2.5	3.4	3.2

To determine optimal settings for minimum or maximum survival, further data reduction was necessary using half factor analysis (10,11). This comparison allows for selection of the optimal setting of the survival parameters based on all possible combinations of parameter settings. Figure 1 displays the difference in bacterial survival between high and low setting for each parameter for each microorganism tested. Bars extending upward or downward from center show the concentration of surviving cells attributable to each parameter. The direction of the bar indicates which setting was favored. For example, extension upward indicates that a greater number of cells survived exposure to the Hi level of a parameter than survived exposure to the Lo level. The height of the bar represents the difference between the number of survivors at the

Hi level versus the Lo level in log CFU/ml. Only data significant within a 95% confidence limit is presented on the graph.

Figure 1 Survivability of 6 Bacteria in 15 Parameter Taguchi Experiment

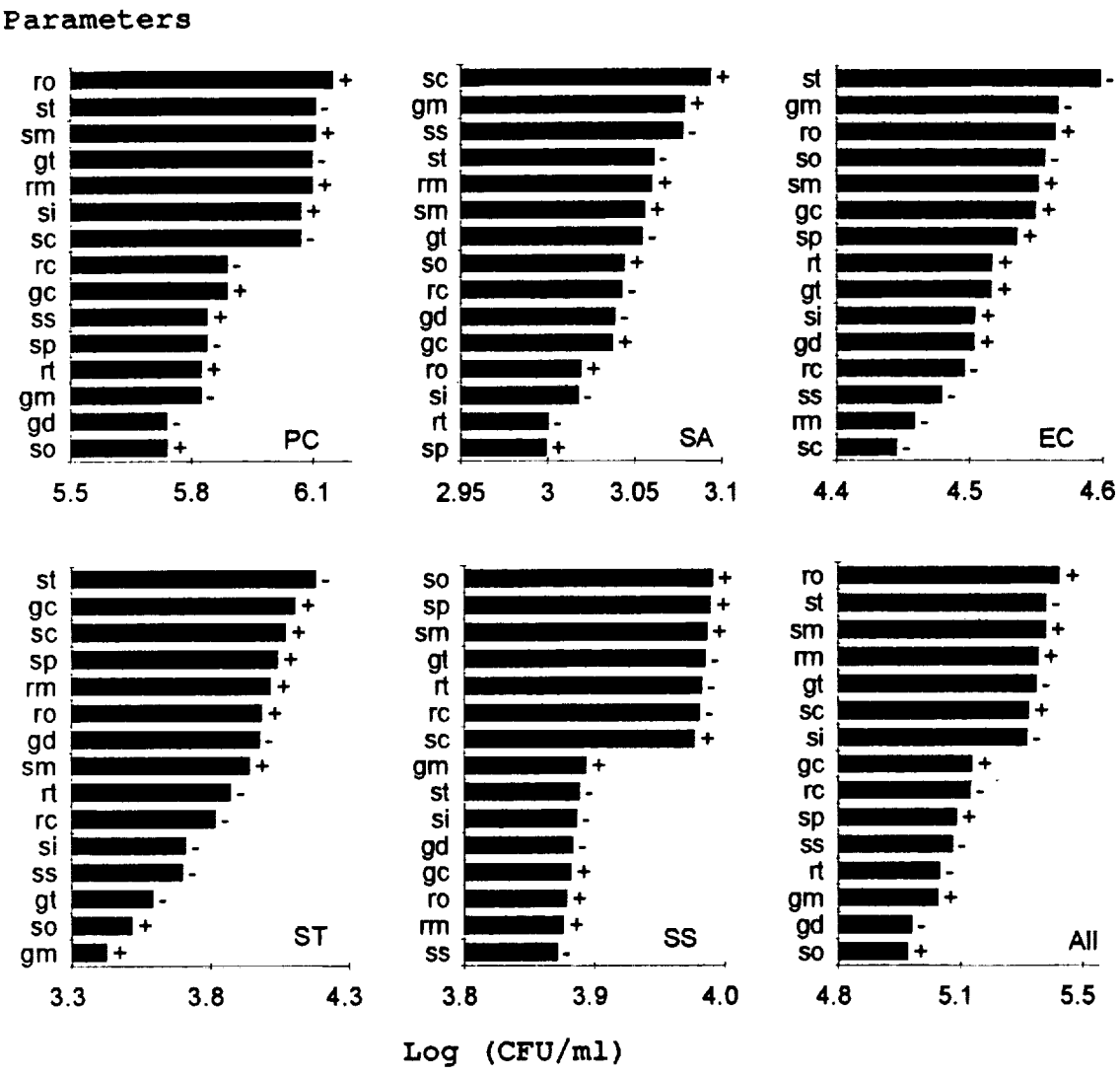


The relative influence of each parameter on bacterial survival is displayed in Figure 2 as six Pareto plots of the half factor analysis. These plots rank the parameters affecting the five surviving microorganisms and the cumulative effect of each parameter on all microorganisms in Log (CFU/ml). The contribution of each parameter is determined from the difference between the average of the Hi and Lo setting of the raw data for each parameter. The magnitude of the difference in survival as a result of each effect is followed by the sign indicating the required setting. Each bar graph displays parameters affecting

survival in order of their significance. Those parameters at the top of the list had the greatest influence on bacterial survival. The last graph shows the effect of each parameter based on the cumulative survival data of all five surviving bacterial species.

Figure 2

Pareto Plots



Because of the overwhelming effect of the high level of survival of *P. cepacia*, the overall Pareto plot is heavily biased.

In order to provide a less biased view of the influence of each parameter on survival, a ranking was made which was strictly based on final order of parameter significance. This ranking of the effects of individual parameters on survival is displayed in Table 6. Each parameter is listed in order of position number with the 15th position producing the greatest survival and the first position the least survival. The set is sorted by average rank to allow the overall effects to be viewed in order of relative importance to microbial survival.

Table 6

Parameter Ranking

	PC	SA	EC	ST	SS	Avg	Std
st	14	12	15	15	7	13	3
sm	13	10	11	8	13	11	2
sc	9	15	1	13	9	9	5
ro	15	4	13	10	3	9	5
gt	12	9	7	3	12	9	3
sp	5	1	9	12	14	8	5
gc	7	5	10	14	4	8	4
gm	3	14	14	1	8	8	5
so	1	8	12	2	15	8	5
rm	11	11	2	11	2	7	4
rc	8	7	4	6	10	7	2
rt	4	2	8	7	11	6	3
si	10	3	6	5	6	6	2
gd	2	6	5	9	5	5	2
ss	6	13	3	4	1	5	4

Predicted settings for optimal bacterial survival were calculated from the data and used to conduct a validation study. Optimum settings for the validation study were derived from calculations of maximum survival for each parameter and are summarized in Table 7. It was assumed that the choice of each optimal individual setting should produce optimum survival if all the parameters were independent. Minimum survival settings were assumed to produce the reverse of the optimal settings.

Table 7

Validation Parameter Settings

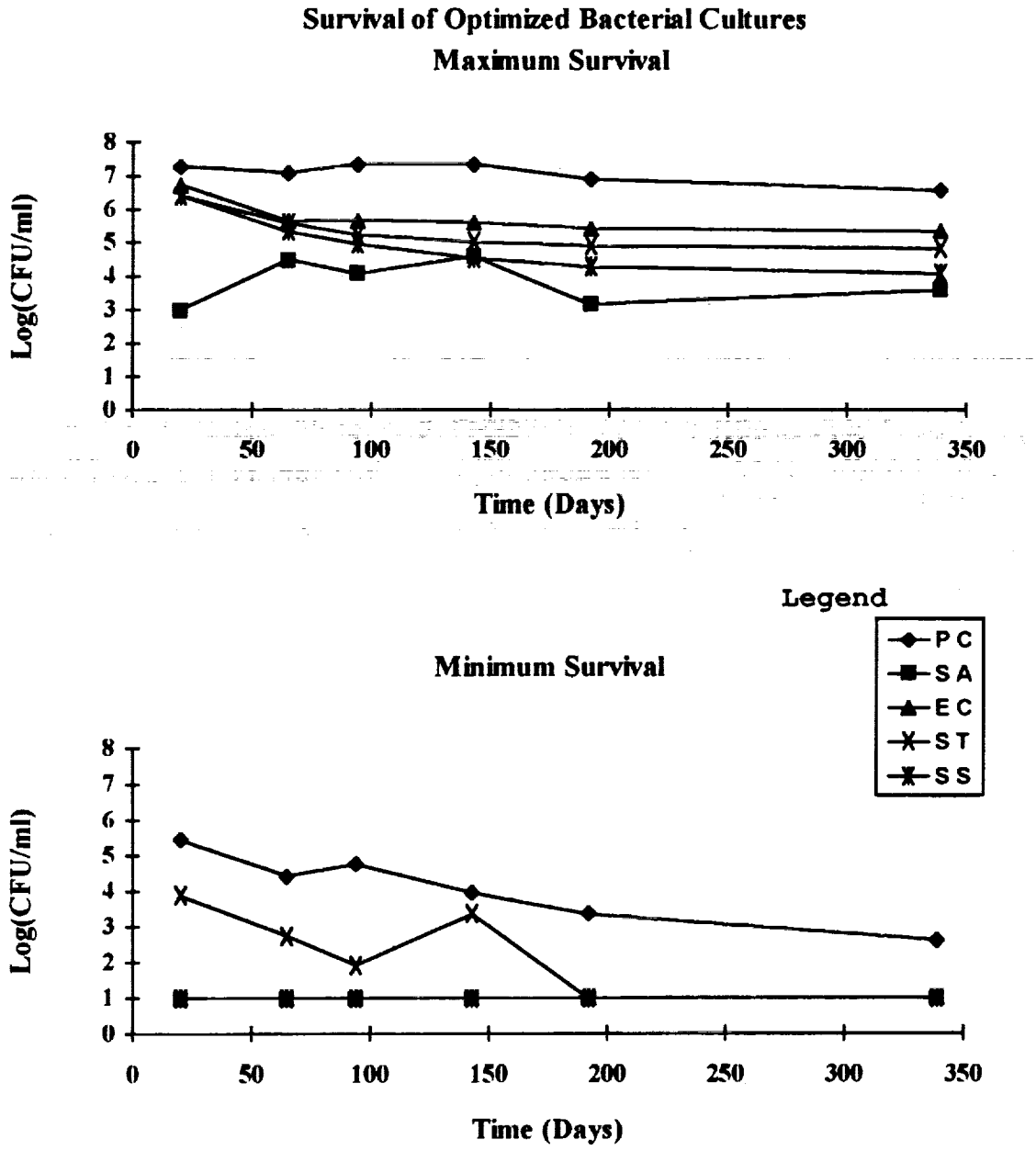
	GM	GC	GT	GD	SM	SC	SS	SP	SI	ST	SO	RM	RC	RT	RO
PC	+	+	-	-	+	+	-	+	-	-	+	+	-	-	+
SA	+	+	-	-	+	+	-	+	-	-	+	+	-	-	+
EC	-	+	+	+	+	-	-	+	+	-	-	-	-	+	+
ST	+	+	-	-	+	+	-	+	-	-	+	+	-	-	+
SS	+	+	-	-	+	+	-	+	-	-	+	+	-	-	+

\+ = Hi setting
\- = Lo setting

Results of the validation test are shown in Figure 3. Number of surviving cells is plotted as log(CFU/ml) versus time for both predicted maximum and minimum settings. Minimum sensitivity of the plate count was a value of 1 log(CFU/ml). There was a significant difference between survival at the maximum and minimum settings. Also, it should be noted that the number of survivors present under optimal conditions exceeded the number of survivors from the initial survival test. Survival numbers appeared to be stable for *P. cepacia, E. coli* and *S. aureus* beginning at day 60 and following, throughout the test. *S. typhimurium* and *S. sonnei* appeared to display a decreasing rate of decline, suggesting a slow approach to stability. No detectable survival occurred at minimal survival settings for *E. coli*, *S. aureus* and *S. sonnei* while *S. typhimurium* decreased to zero during the first 200 days and *P. cepacia* continuously decreased during the entire course of the experiment.

A significant difference in survival was found between bacteria in sub-optimal conditions (ddH2O) during initial survival testing and bacteria under optimal conditions (Table 8). An increase in survival of several orders of magnitude was observed under optimal conditions. Similar results were seen for minimum survival conditions.

Figure 3


Survival of Optimized Bacterial Cultures
Maximum Survival
Log(CFU/ml)
Time (Days)
Legend
PC
SA
EC
ST
SS
Minimum Survival
Log(CFU/ml)
Time (Days)

DISCUSSION:

Preliminary long term survival studies demonstrated that aquatic bacteria can survive 15 months in deionized distilled water. Scanning electron micrographs showed a size reduction of these bacteria and a variable morphology after starvation.

Taguchi optimization of the effect of 15 parameters on bacterial survival demonstrated that specific conditions can significantly alter the length of time bacteria can persist in aquatic environments. Validation testing of predicted optimal survival conditions demonstrated that not only was survival as high as seen in preliminary comparative testing but that survival could be increased by optimizing experimental conditions.

Table 8

Survival comparison
Log (CFU/ml)

Experiment	Month	PC	SA	EC	ST	SS
Initial survival test	15	6.25	<1	4.57	2.87	2.74
Optimized survival	12	6.56	3.60	5.33	4.82	4.08

Analysis of survival parameters from the Pareto plots in Figure 2 demonstrated that each bacterial type preferred a unique set of conditions for optimal survival. Several parameters appeared to produce a consistent improvement in survival while a separate set showed a lesser effect on survival. The parameter having the most significant effect on the survival of *P. cepacia* was oxygen concentration in the recovery environment (RO). *P. cepacia* is a strict aerobe and therefore could not be recovered when incubated anaerobically. This is a well established effect and served primarily as a positive control demonstration on the reliability of the results. In contrast, oxygen concentration was the least significant parameter during the period in the aquatic environment. This effect may reflect the absence of a need for oxygen during the period of aquatic survival and minimal metabolic activity.

Analysis of the overall effects on the combined survival of all five bacterial species cannot be judged fairly by the cumulative Pareto plot due to the bias resulting from the significantly higher number of *P. cepacia* survivors. The influence of the individual parameters on each bacterial species can most easily be summarized from the rank ordering of the parameters. The comparison based on ranking alone (Table 6) provides a more realistic analysis of the overall significance of the tested parameters on bacterial survival in this experiment due to the differing survival values for each species.

The parameter holding a high rank most frequently and with greatest consistency was survival temperature (ST). A lower temperature (25C) produced the greatest survival for all but *E. coli* which preferred the higher temperature (35C) for survival. The next two major influences on survival were medium type (SM) and concentration (SC). The complex nutrients of BHI promoted survival in the enteric bacteria while minimal medium enhanced survival of *P. cepacia* and *S. aureus*. Low concentrations of either medium enhanced survival in all but *S. aureus* which required the higher concentration. Salinity (SS) and pH (SP) appeared to have a lesser influence on survival as individual parameters, but because of their uniform effect on all bacterial species tested, survival could be reduced by higher concentration of salinity and lower pH.

Recovery conditions in general (RM, RC, RT) had some of the weakest effects on the survival data except for recovery oxygen concentration (RO). It can be concluded that if an appropriate medium is used for the recovery of a known species, and incubation conditions are appropriate, recovery is not significantly altered.

Further analysis of the influence of individual parameters for each species did not reveal any particular pattern. The effect of the other parameters was quite varied and no generalizations are readily apparent. This may be explained by the fact that each species is native to a unique set of environmental conditions and possesses its own set of attributes enabling it to withstand various environmental stresses.

In summary, for practical application of this information, to maintain the highest water quality with minimum microbial contamination in a water reclamation system, a processing system should be adjusted to provide the maximum temperature and salinity at the lowest pH within acceptable limits. At the same time, a minimal nutrient level (i.e., total organic carbon - TOC) should be maintained since complete elimination of all organics compounds appears to facilitate long term survival mechanisms. In an earlier study, the reduction of total organic carbon content of water to less than 0.5 ppm may have actually increased the survival rate of microorganisms in the water. Potentially, higher TOC content could increase

microbial susceptibility to disinfectant, reducing the total amount of disinfectant necessary to guarantee safe potable water, and reducing the amount of processing and cost necessary to produce water. Further testing should be performed on specific sets of environmental conditions.

Though the Taguchi approach is a relatively new method of experimental design for microbial ecology, these results offer the potential for major improvements in the methods of microbial control in aquatic environments.

This information will be useful in predicting the optimal survival conditions for each type of bacterium tested and determining which species may dominate under various conditions of a water reclamation system. With this information, disinfection methods can be tested for most effective microbial control as a means to minimize health risks and microbially influenced corrosion in a water system.

REFERENCES

1. Rodgers, E. B. 1986. The Ecology of Microorganisms in a Small Closed System: Potential Benefits and Problems for Space Station. NASA Technical Memorandum, TM-86563.

2. Bagdigian, R. M., M. S. Traweek, G. K. Griffith, and M. R. Griffin, Phase III Integrated Water Recovery Testing at MSFC: Partially Closed-Hygiene Loop and Open-Potable Loop Results and Lessons Learned, Presented at the 21st Intersociety Conference on Environmental Systems, San Francisco, CA, 1991.

3. Roman, M.C., J. Gauthier, M.E. Wilson, D.W. Terrell, T.L. Huff and D.C. Obenhuber. 1991. Microbial Distribution in NASA Environmental Control Life Support System: Water Recovery Test for Space Station Freedom. International Conference on Environmental Systems. Report #91277

4. Obenhuber, D.C., T.L. Huff and E.B. Rodgers. 1991. Microbial Biofilm Studies of the Environmental Control and Life Support System Water Recovery Test. International Conference on Environmental Systems. Report #91278

5. Roszak, D. B. and R. R. Colwell. Survival Strategies of Bacteria in the Natural Environment. Annual Review of Microbiology 51, 365-379, 1987.

6. Obenhuber, D.C., R.D. Taylor, R.J. Bruce, D.L. Pierson and E.B. Rodgers. 1991. Microbial Starvation Survival of Nine Bacterial Cultures for Over 1 Year in Distilled Deionized Water. American Society of Microbiology, Dallas, TX

7. Carson, L.A., M.S. Favero, W.W. Bond, and N.J. Petersen. 1973. Morphological, Biochemical, and Growth Characteristics of Pseudomonas cepacia from Distilled Water. Appl. Microbiol.

25:476-483.

8. Novitsky, J.A. and R.Y. Morita. 1976. Morphological Characterization of Small Cells Resulting from Nutrient Starvation of a Psychrophilic Marine Vibrio. Appl. Environ. Microbiol. 32:617-622

9. Morita, R.Y. 1985. Starvation and Miniaturization of Heterotrophs, with special Emphasis on Maintenance of the Starved Viable State in Bacteria in Their Natural Environments, Fletcher, M. and G. Floodgate (eds.). pp.111-129. Academic Press, New York

10. Schmidt, S.R and R.G. Laundsby. 1989. Understanding Industrial Designed Experiments. CQG Ltd. CO.

11. Kiemele, M.J. and S.R. Schmidt. 1990. Basic Statistics. Air Academy Press. Colorado Springs, CO

APPROVAL

OPTIMIZATION OF 15 PARAMETERS
INFLUENCING THE LONG TERM SURVIVAL
OF BACTERIA IN AQUATIC SYSTEMS

By Donald C. Obenhuber

The information in this report has been reviewed for technical content. Review of any information concerning Department of Defense or nuclear energy activities or programs has been made by the MSFC Security Classification Officer. This report, in its entirety, has been determined to be unclassified.

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