**Study Title**

The Efficacy of BromMax (EPA Reg. No. 63838-2) Against a Consortium of Oil an Gas Field Bacteria including General Aerobic Bacteria (GAB), Acid Producing Bacteria (APB), and Sulfate Reducing Bacteria (SRB)

**Data Requirement**

Efficacy Requirements to Obtain CDPR Registration for Registration of BromMax as an Oil and Gas Field Microbiocide

**Performing Laboratory**

Scott Campbell MSc; Angel Johnson MSc
Intertek Commercial Microbiology
600 Port West Drive
Houston TX 77024

**Authors**

Jonathan Howarth Ph.D; Courtney Mesrobian B.Sc.
Enviro Tech Chemical Services
500 Winmoore Way
Modesto, California

**Sponsor**

Enviro Tech Chemical Services
500 Winmoore Way
Modesto, CA 95358

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**STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS**

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10(d)(1)(A), (B) or (C)

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| Company | Enviro Tech Chemical Services Inc. |
| Company Agent | Michael S. Harvey |
| Title | President and CEO |
| Signature | ________________________________ |
| Date signed | ______________________ |

**GOOD LABORATORY PRACTICE STATEMENT**

This report, entitled "The Efficacy of BromMax (EPA Reg. No. 63838-2) Against a Consortium of Oil and Gas Field Bacteria including General Aerobic Bacteria (GAB), Acid Producing Bacteria (APB), and Sulfate Reducing Bacteria (SRB) is a discussion and presentation of information. This study was not conducted in compliance with EPA Good Laboratory Practice Standards (40 CFR Part 160) for the following reason:

There was no QAU review of Test Protocol, In-Phase Study conduct or final report.

Study Director: ______________________ Date signed: ___________
Jonathan Howarth, PhD
Senior VP Technology
Enviro Tech Chemical Services

Submitter: ______________________ Date signed: ___________
Michael S. Harvey
CEO
Enviro Tech Chemical Services

Sponsor: Enviro Tech Chemical Services

Sponsor Representative: ______________________ Date signed: ____________
Michael S. Harvey
CEO
Enviro Tech Chemical Services

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## Background

The California Department of Pesticide Regulation (CDPR) requires the submission of efficacy data in order to acquire a California registration. Enviro Tech is pursuing CDPR approval for BromMax use in the gas and oil industries. Therefore efficacy testing was performed by an independent third party testing facility, Commercial Microbiology, Inc.. However, the report does not clearly identify the chemical being referred to. Nor does it present data in graphical terms and interpret the data for the reader. This report, authored by the co-sponsor of the study is designed to make up for these shortcomings.

## Methodology

### Planktonic and Sessile (Biofilm) Biocide Efficacy

Commercial Microbiology Inc. was provided with water samples, similar to the water used in oilfield systems, to be used in the requested efficacy testing. Commercial Microbiology Inc. performed water chemistry analysis, which included Total Suspended Solids (TSS), Total Organic Carbon (TOC) and Total Dissolved Organic Carbon (TDOC). These tests were completed to determine if there was enough Carbon in the water samples to support the bacteria growth. The water samples provided were used as the matrix for the efficacy testing.

### Test Bottle and Bacteria Preparation

An equal amount of source water was distributed into 10 bottles. The pH of the source water was measured immediately preceding the start of the experiment. The addition of the biocides may have changed the pH of the source water, but no pH adjustments were made through the course of the experiments to insure that the tests were performed according to field procedures. Prior to biocide introduction, corrosion coupons were placed in the bottom of the bottles as a removable surface to assess sessile bacteria accumulation (biofilm).

The base stock culture consisted of General Aerobic Bacteria (GAB), Acid Producing Bacteria (APB), and Sulfate Reducing Bacteria (SRB). It was created by inoculating 9 mL of fresh respective bacterial growth media with 1mL of the respective bacterial consortium. The inoculated stock cultures were incubated at 35$^{o}$C for 2-4 days to revive the bacteria and promote the log phase of growth.

The bacteria described above were used for the inoculation. The base stock culture was spun down and washed in Phosphate Buffered Saline (PBS) buffer to exclude the respective media, which was used to revive the inoculums. The bacteria were then re-suspended in PBS aliquots that were used for the inoculation of the neat test waters for the BromMax evaluation. Each test bottle was inoculated such that approximately $10^6$ population of each GHB, APB, and SRB was achieved. Test bottles were then allowed to stabilize for four hours prior to the addition of the biocide.

Evaluation of BromMax

Five test bottles were set up to evaluate BromMax at four different concentrations, which were 10, 25, 50, and 100 ppm as measured residual bromine with one control. The minimum dosage of BromMax required to obtain the four desired residual concentrations was determined prior to the experiment. Four samples were dosed and the desired residual concentrations were maintained (10 ppm, 25 ppm, 50 ppm, and 100 ppm as bromine) for 30 minutes. After the 30 minutes, the maintenance of the bromine residual was terminated. Before each sampling time, the test bottles were mixed vigorously to insure re-suspension and equal distribution of bacteria. At 0 min, 5 min, 30min, 4 hr, and 28 days, the surviving planktonic SRB, GHB, and APB were enumerated utilizing the Most Probable Number (MPN) method. At 4 hr and 28 days, the surviving sessile SRB, GHB, and APB were enumerated utilizing the same method. The GHB, APB and the SRB were incubated for 28days at $30^{o}$C. The enumeration process was completed following the NACE TMO 194-94 recommendations for microbial monitoring in oilfield systems.

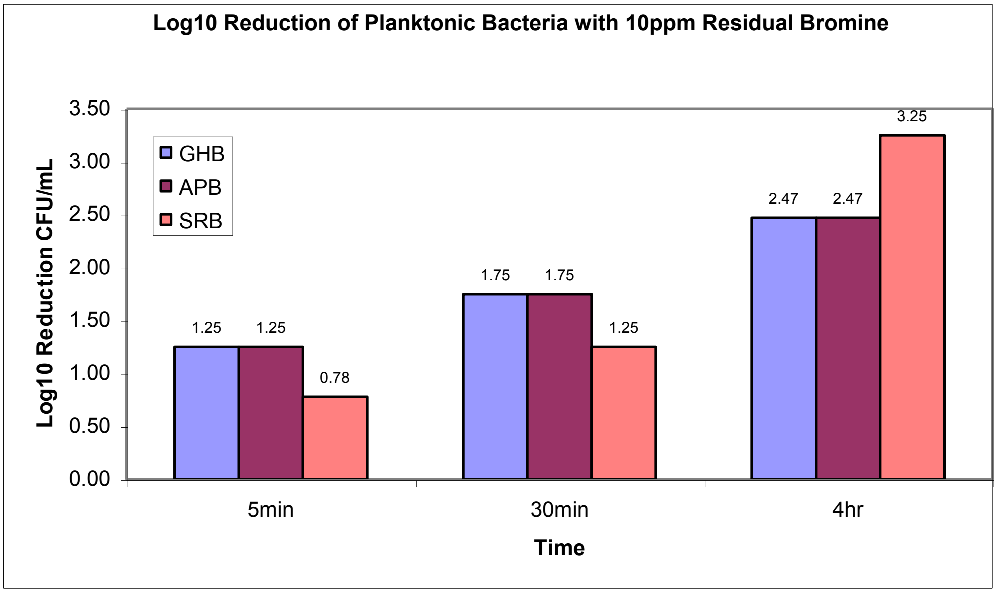
## Results/Discussion

Phase 1 utilized a primary treatment of BromMax. This biocide was tested at four different concentrations, 10, 25, 50, and 100 ppm as residual bromine, along with a control. Two types of bacteria were tested, Planktonic and Sessile bacteria. The bacteria was tested separately because Planktonic bacteria suspends itself in the fluid while the sessile bacteria settle out of the fluid and attached to the surface of the corrosion coupons. BromMax is utilized as a fast, initial disinfectant. This was the primary treatment in Phase 1 testing. Graphs 1a-4a display only the first four hours of treatment due to the fact that BromMax provides very little residual disinfectant beyond four hours.

Graphs 1a-4a compare the $\log_{10}$ reductions of Planktonic bacteria (GHB, APB, and SRB) at 5min, 30min, and 4hrs in the water samples before and after a primary treatment with BromMax at the four different concentrations (10, 25, 50, and 100 ppm as residual bromine).

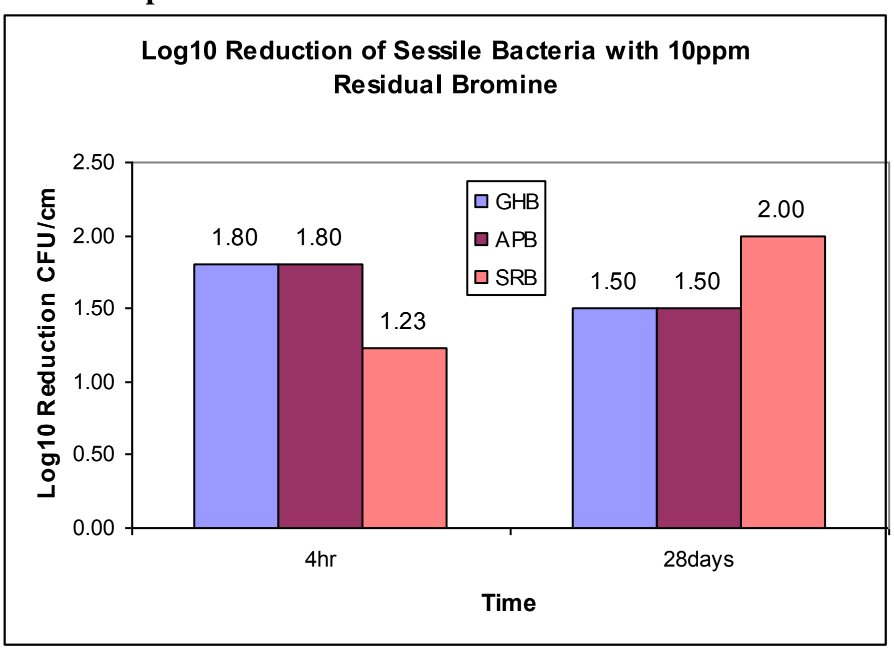
Graph 1a illustrates the $\log_{10}$ reduction of planktonic bacteria with a treatment of BromMax at 10 ppm as residual bromine. This treatment has the lowest concentration of bromine, but still offers disinfectant properties against the planktonic bacteria. The $\log_{10}$ reduction at five minutes is 1.25 for GHB and APB, but only 0.78 for SRB. As can be seen in graph 1a, the $\log_{10}$ reduction continually increases over the four-hour treatment period.

**Graph 1a**



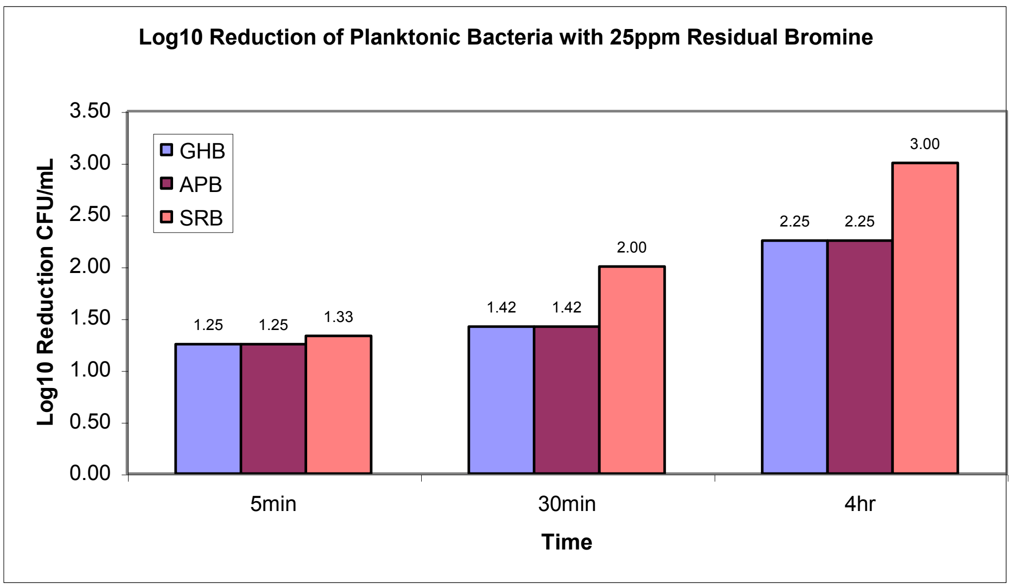

Graph 1b illustrates the $log_{10}$ reduction of sessile bacteria (GHB, APB, and SRB) at four hours and 28 days. The trend in $log_{10}$ reductions displayed in graph 1b indicates that over time the sessile GHB and APB regrow. This is to be expected with only a primary treatment of BromMax at such a low bromine concentration when compared to the treatments of higher bromine residual.

**Graph 1b**

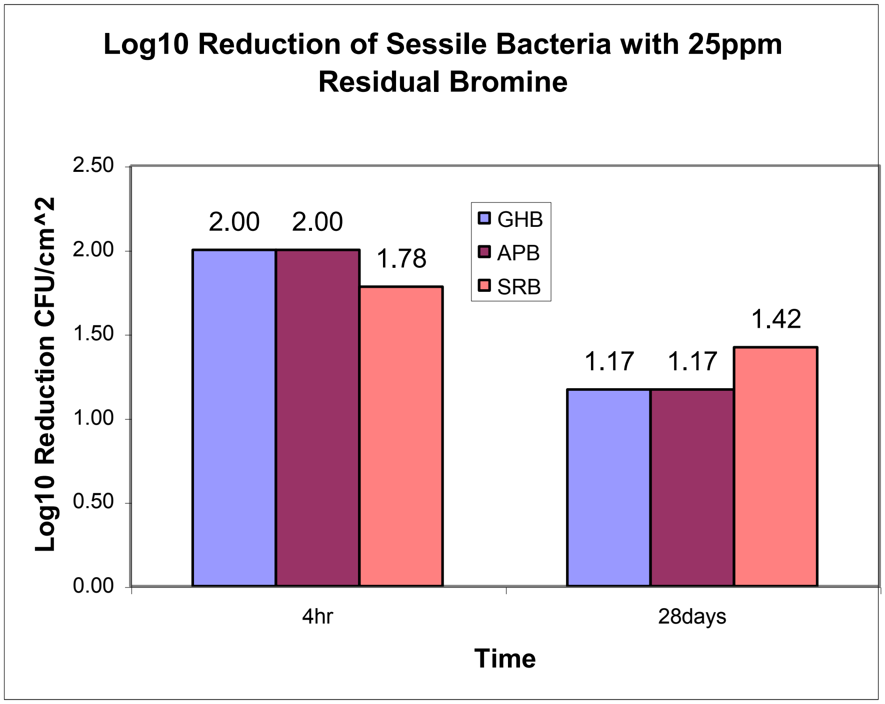


Graph 2a illustrates the $\log_{10}$ reduction of planktonic bacteria with a treatment of BromMax at 25 ppm as residual bromine. This treatment has a low concentration of bromine, but offers greater disinfectant qualities against the planktonic bacteria than the 10 ppm treatment. The $\log_{10}$ reduction at five minutes is 1.25 for GHB and APB, but only 1.33 for SRB. As can be seen in graph 2a, the $\log_{10}$ reduction continually increases over the four-hour treatment period. Graph 2b illustrates the $\log_{10}$ reduction of sessile bacteria (GHB, APB, and SRB) at four hours and 28 days. The trend in $\log_{10}$ reductions displayed in graph 2b indicates that over time all three types of the sessile bacteria experience some regrowth. This is to be expected with only a primary treatment of BromMax at such a low concentration.

**Graph 2a**

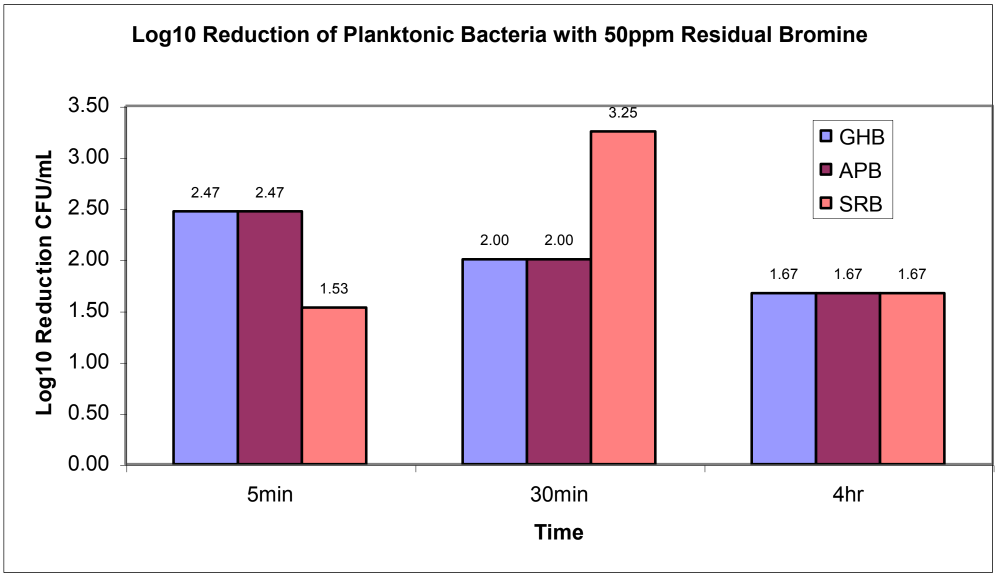


**Graph 2b**

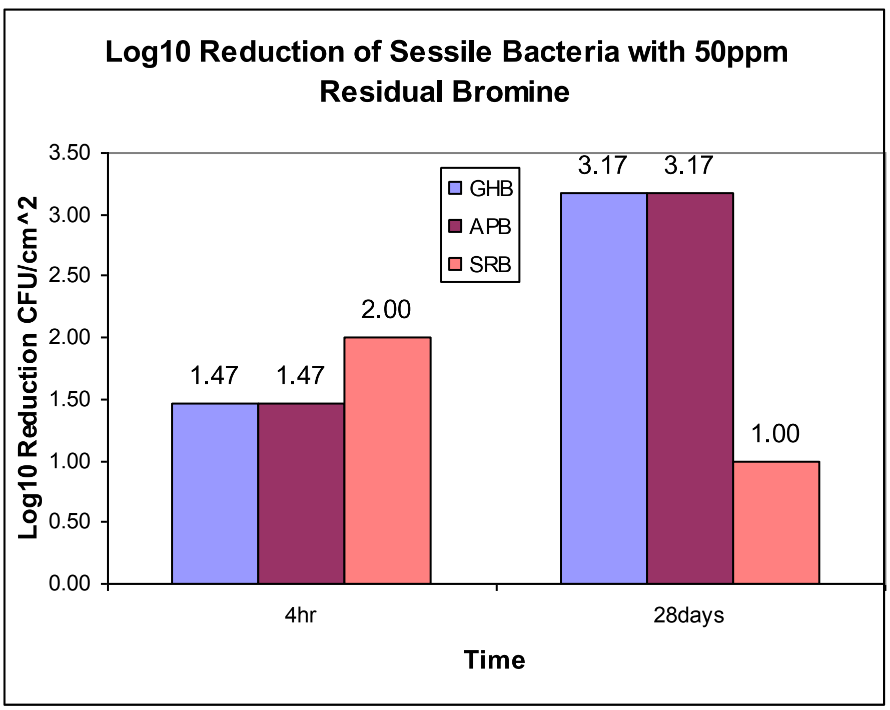



Graph 3a illustrates the $\log_{10}$ reduction of planktonic bacteria with a treatment of BromMax at 50 ppm as residual bromine. The $\log_{10}$ reduction at five minutes is 2.47 for GHB and APB, but only 1.53 for SRB. As can be seen in graph 3a, the $\log_{10}$ reduction continually decreases over the four-hour treatment period. Graph 3b illustrates the $\log_{10}$ reduction of sessile bacteria (GHB, APB, and SRB) at four hours and 28 days. The trend in $\log_{10}$ reductions displayed in graph 3b illustrates that over time the 50 ppm concentrated treatment was effective at reducing GHB and APB up to 28 days, but the SRB rebounded after 28 days.

**Graph 3a**

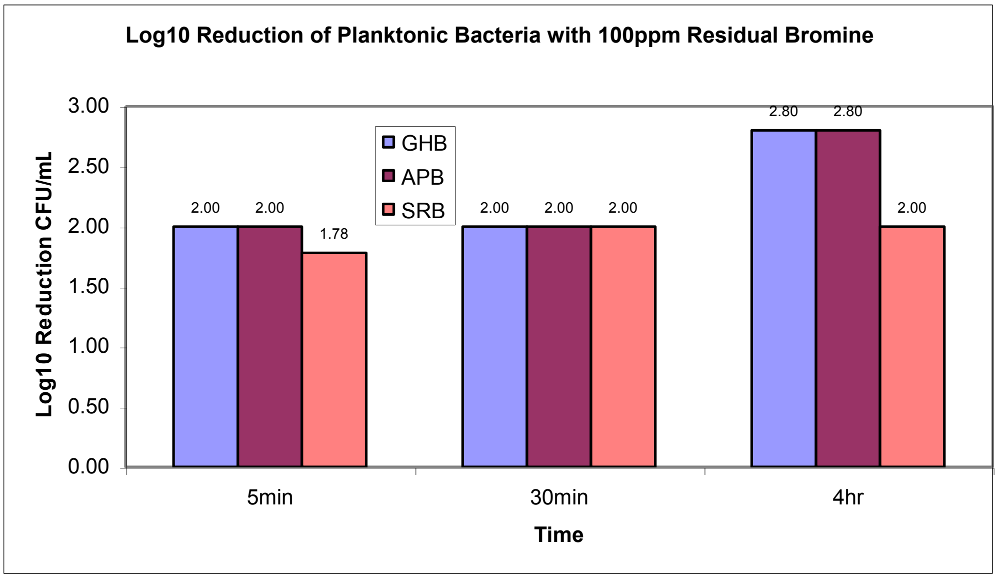



**Graph 3b**

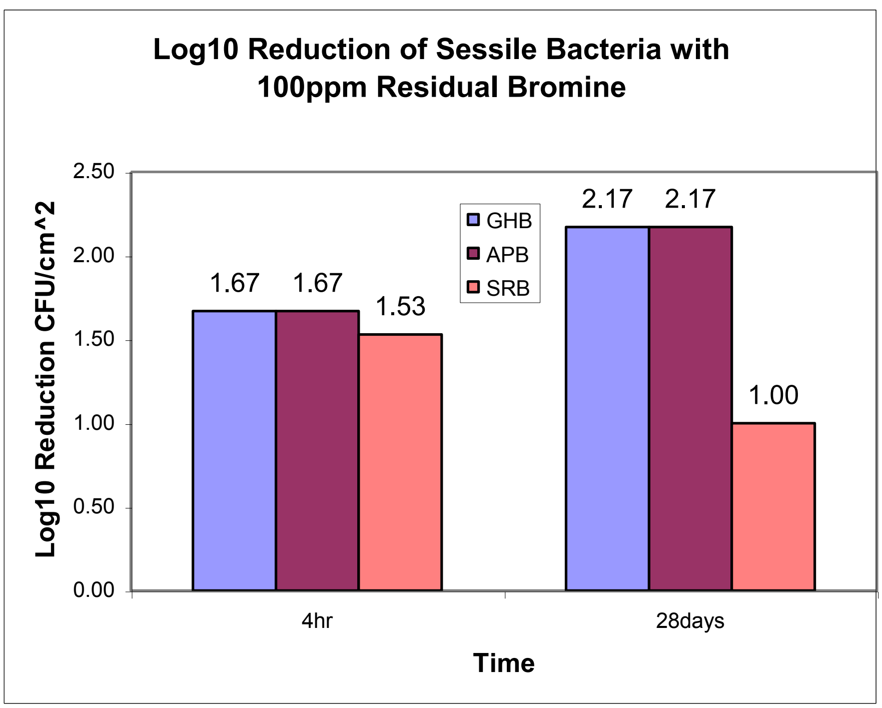


Graph 4a illustrates the $log_{10}$ reduction of planktonic bacteria with a treatment of BromMax at 100 ppm as residual bromine. The $log_{10}$ reduction at five minutes is 2.00 for GHB and APB, but only 1.78 for SRB. As can be seen in graph 4a, the $log_{10}$ reduction is stable over the four-hour treatment period. Graph 4b illustrates the $log_{10}$ reduction of sessile bacteria (GHB, APB, and SRB) at four hours and 28 days. The trend in $log_{10}$ reductions displayed in graph 4b illustrates that over time the 100 ppm bromine treatment was effective at reducing GHB and APB up to 28 days, but the SRB rebounded after 28 days.

**Graph 4a**

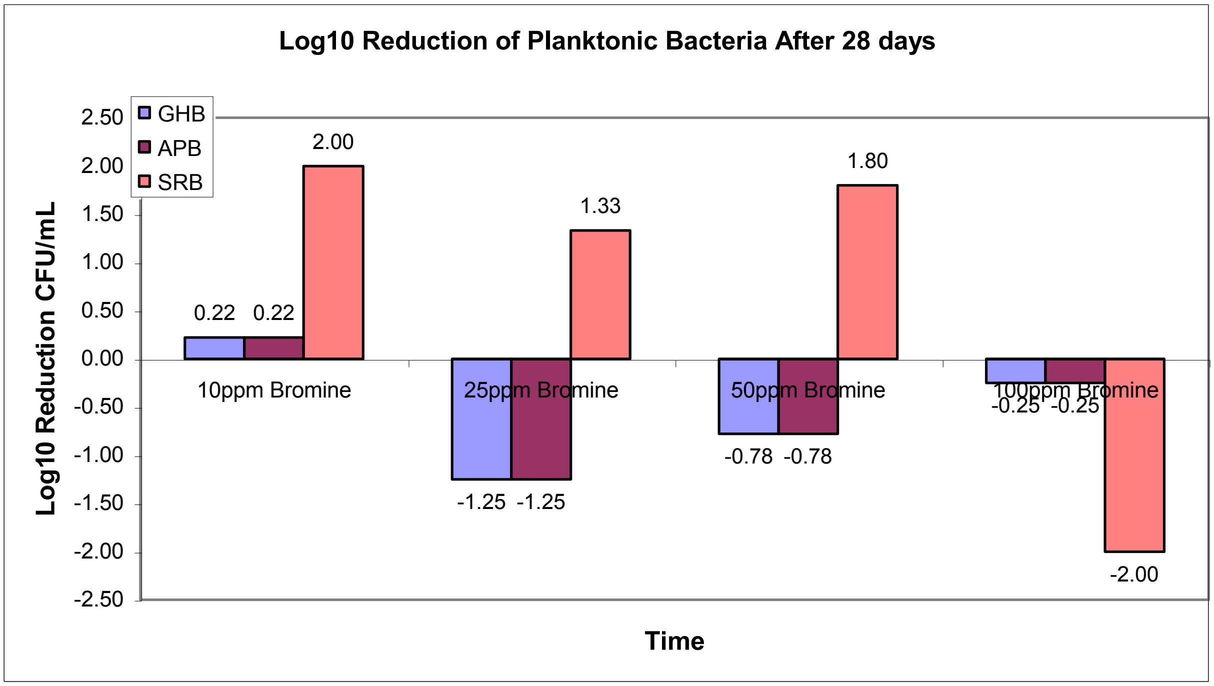



**Graph 4b**



Graph 5 illustrates the rebound in GHB, APB, and SRB after 28 days for the four different treatments of BromMax. It is expected that some bacteria would rebound after 28 days. For the treatments of 10, 25, and 50 ppm bromine only the SRB showed a substantial rebound. The treatment with 100 ppm bromine showed no rebound of any bacteria. This is not surprising given the high concentrated dose that was applied.

**Graph 5**




## Conclusion

- Enviro Tech is seeking to obtain a California registration for BromMax in the gas and oil industries. Efficacy testing was performed at an independent third party testing facility, Commercial Microbiology, Inc.
- Commercial Microbiology Inc. was provided with water samples, similar to the water used in oilfield systems, to be used in the required efficacy testing. The water samples provided were used as the matrix for the efficacy testing.
- General Aerobic Bacteria (GAB), Acid Producing Bacteria (APB), and Sulfate Reducing Bacteria (SRB) were used in this efficacy study. These bacteria consisted of planktonic and sessile bacteria; therefore two sampling methods were utilized.

- At the lower concentrations of 10 and 25 ppm as bromine, all three sessile consortia of bacteria experienced a slight regrowth over a 28-day incubation period. However, none of the bacterial had had entered the exponential growth phase. At the higher concentrations of 50 and 100 ppm as bromine sessile APB and GAB were effectively controlled for the entire 28-day incubation period whereas SRB were starting to regrow slightly.
- 10 ppm and 25 ppm bromine from a primary treatment of BromMax provided excellent disinfectant qualities against planktonic and sessile GAB APB and SRB consortia of microorganisms over relatively short contact times (30 minutes to four hours). Not surprisingly, higher concentrations of bromine (50 ppm and 100 ppm) provided even better biocide disinfectant performance and also conferred better long-term properties against bacterial rebound. However, the improvement in performance is probably not worth the additional cost of administering higher doses of bromine from BromMax.
- The results presented in this study indicate that a shock dose of 10 ppm as bromine from BromMax would be suitable for the treatment of any water system where planktonic and sessile GAB, AHP and SRB consortia are problematic. Moreover, this level of BromMax confers adequate long-term performance on sessile (biofilm) bacteria, because following the initial dose, none of the microorganisms had entered the exponential growth phase during a 28-day incubation period.