BENCH SCALE STUDIES TO EVALUATE THE EFFECTIVENESS OF SEVERAL BIOCIDES AND CHEMICALS WITH COMPARISONS TO FACTORY TRIALS

By Indrani Samaraweera, Gary Fischer, Diane Rheault, Bill Colonna, and Lynn Buschette

INTRODUCTION
Biocides have been in use in the sugar industry for many years. However, evaluating the effectiveness of biocides in the factory alone is difficult due to other factory operations (other additives, temperature, etc.) interfering with results. Therefore, several bench scale studies were carried out from March to May 1999 with the use of raw juice prepared in the pilot plant at American Crystal Sugar Company's Technical Services Center without the use of additives. A number of biocides (Kcide-800, Tsunami-100, Kcide-850) and chemicals (sodium sulfite, sulfur dioxide, and ammonium bisulfite) were evaluated at different concentrations to determine biocidal efficacy on microbial populations. These studies were followed by a trial with use of these biocides at the Crookston factory.

MATERIALS & METHODS
A) BIOCIDES – Three different biocides were evaluated.
   1. Kcide-800 (Kabo Chemicals) with a specific gravity of 1.176 – The active ingredients being sodium dimethyldithiocarbamate (15-16%), and Nabam or disodium ethylene-bisdithiocarbamate (15-16%). This biocide was evaluated at 20, 40, 100, and 400 ppm (and with corrections for specific gravity it was evaluated at 23.52, 47.04, 117.6, and 470.4 ppm respectively).
   2. Tsunami-100 (Ecolab) with a specific gravity of 1.11 – The active ingredients being peroxyacetic acid (15%) and hydrogen peroxide (11%). This biocide was evaluated at 5.0 and 80 ppm concentrations (and with corrections for specific gravity at 5.55 and 88.8 ppm respectively).
   3. Kcide-850 (Kabo Chemicals) with a specific gravity of 1.129 – The active ingredient being glutaraldehyde (50%). This biocide was evaluated at 20, 250, and 500 ppm (and with corrections for specific gravity at 22.58 ppm, 282.25 ppm, and 564.5 ppm respectively).

B) CHEMICALS – Three different chemicals were evaluated.
   1. Sodium sulfate (Na₂SO₃) – This was evaluated at 167.6 and 327.5 ppm concentration on titration or 200 and 400 ppm concentration on weight basis.
   2. Ammonium bisulfite (NH₄HSO₃), 45% solution with a specific gravity of 1.25 – This was evaluated at 200, 400, 600, and 800 ppm concentrations (and with corrections for specific gravity at 249.75, 499.5, 749.25, and 999.0 ppm respectively).
   3. Sulfur dioxide (SO₂) – This was sparged into raw juice as close to 200 and 400 ppm as possible and evaluated at 195.5 and 363.8 ppm concentrations.

C) EXPERIMENTS – A number of bench scale biocide studies were carried out with Kcide-800 (5 studies), Tsunami-100 (3 studies), Kcide-850 (3 studies), sodium sulfate – Na₂SO₃ (1 study), Sulfur dioxide – SO₂ (1 study), and ammonium bisulfite – NH₄HSO₃ (2 studies). The initial
Kcide-800 and Tsunami-100 studies were preliminary studies to find suitable parameters for further studies. Therefore only the latter studies with similar parameters will be used in main discussions with minor references to other experiments.

1. Raw Juice – The raw juice was prepared at the Technical Services Center pilot plant without the use of any additives on 1/8/99 and used in all evaluations. This raw juice was poured into one gallon containers and kept frozen in a walk-in freezer at -15°F. Single gallon containers of juice were thawed out for ≈1¼ days and used as required for experiments. The time of 1¾ days for thawing and storing of juice at ambient temperature in the lab (≈25°C) was found to give a sufficient microbial level (mesophiles log 4.5 to 5.0 cfu/g and thermophilic log 3.0 cfu/g) for biocide evaluations. In other preliminary studies the raw juice was thawed and stored for shorter and longer periods of time (1, 2½, 3¼, 4¼ days).

2. Time and Duration for Incubation of Sample Bottles – Sample bottles with different concentrations of biocides and control bottles (without biocide) were incubated at 40°C for 5 hours. In a few preliminary trials test samples were incubated at 25°C for 30 minutes.

3. Microbial Counts – Initial mesophilic and thermophilic counts in raw juice after thawing at the start of experiment (before addition of biocide or incubation of bottles) was obtained. This was followed by obtaining another set of microbial counts (mesophiles, thermophiles) in control bottle (no biocide) and other biocide treated sample bottles after incubation of the bottles for 5 hours at 40°C or 30 minutes at 25°C during initial experiments. The raw juice samples were evaluated for yeast and mold as well, but these organisms were found to be absent in all the raw juice experiments after processing and freezing.

D) BIOCIDE TRIALS AT ACS FACTORY

- This trial was carried out at one of the ACS factories for five days from 4/29 through 5/3/99. Samples for microbial evaluations (mesophiles, thermophiles, Leuconostoc, yeast and mold) were taken from five locations in the factory. These were: diffuser cell 1, diffuser cell 5, raw juice surge tank, raw juice before heater, and the pulp press water tank.
- Further factory trials with addition of Kcide-800 before and after the slicer are in progress.

RESULTS AND DISCUSSION

The controlled experimental conditions of thawing raw juice for 1¾ days and incubation of control and biocide treated bottles at 40°C for 5 hours were carried out for the following:

- Kcide-800 (thiocarbamate) – Two experiments carried out on 3/30/99 and 4/7/99, respectively.
- Tsunami-100 (H₂O₂ and per acetic acid) – Two experiments carried out on 3/24/99 and 6/9/99, respectively.
• Kclide-850 (glutaraldehyde) – Two experiments carried out on 5/12/99 and 6/9/99, respectively.

In addition, single experiments with the above controlled conditions were carried out for SO₂ with samples of raw juice on 4/7/99 and sodium sulfite (Na₂SO₃) on 4/7/99. Also two experiments were carried out for ammonium bisulfite on 5/12/99 and 7/28/99. See summary of results for these studies in Table 1 attached.

In the case of thiocarbamate (Kclide-800) the mesophilic count showed a decrease of 3 log units and thermophilic counts, a decrease of 2 log units at 23.52 ppm and 47.04 ppm as compared to control when incubated at 40°C for 5 hours. These results were the same in both experiments carried out on 3/30/99 and 4/7/99.

The Tsunami-100 was evaluated at concentrations of 5.55 ppm and 88.8 ppm. This biocide was found to have no effect on mesophilic and thermophilic populations at ≈5 ppm concentration in both experiments of 3/24/99 and 6/9/99. However, the 88 ppm concentration showed greater than 1 log unit decrease in mesophilic counts and 1.5 log unit decrease in thermophilic counts as compared to control in experiments of 3/24/99. However, in the second experiment (6/9/99) the 88 ppm concentration of Tsunami-100 had no effect on mesophiles and showed 0.5 log unit decrease in thermophilic count.

In the case of Kclide-850 (glutaraldehyde), this showed a decrease of mesophilic counts of greater than 3 log units and decrease of thermophilic counts of 1.5 log units at 22.58, 282.25, and 564.5 ppm concentration in experiment on 5/12/99. In the second experiment on 6/9/99 only a concentration of 22.58 ppm which is close to the maximum concentration permitted for use (20 ppm) was evaluated. Here mesophilic counts decreased by greater than 4 log units and thermophilic counts decreased by one log unit at a concentration of 22.58 ppm.

However, interestingly in preliminary Kclide-800 experiments (3/15/99, 3/16/99, 3/22/99) and preliminary Tsunami-100 experiment (3/17/99) when raw juice was allowed to thaw and be kept at ambient temperatures for 2½ to 4½ days giving initial mesophilic counts in log 7.8 to 8.5 range and thermophilic counts in the log 3 cfu/g range. (See Table 2) No significant decrease in mesophilic or thermophilic counts at 23.52, 47.04, 117.6, and 470.4 ppm of Kclide-800 or 5.5 and 88.8 ppm concentration of Tsunami-100 was observed. This is probably due to the fact that the microbial loading of mesophiles (log 7.8 to 8.5 cfu/g) and thermophiles (log 3 cfu/g) are too high for the biocide to have any impact on decreasing numbers of microbes. It is also probably due to the fact that the microbes have reached the stationary phase of growth in the life cycle where the number of cells produced is equal to the numbers dying off giving a flat curve. This phase of growth is also probably less susceptible to biocidal activity unlike the growth phase where there is active multiplication of cells with increase in numbers.

In the bench scale studies with chemicals containing SO₂ the following was observed. See Table 1 for detail. For instance in the sparged SO₂ experiment of 4/7/99 the mesophilic counts decreased by >2.5 log units and thermophilic counts by >1 log unit at 195.5 ppm concentration as compared to the control. Also at a higher concentration of 363.8 ppm SO₂ the mesophilic
counts decreased by 3 log units and thermophilic counts decreased by >1 log unit (which was similar to the result at 195.5 ppm concentration).

Na$_2$SO$_3$ was less effective than sparged SO$_2$. See Table 1 for detail. For instance in the experiment carried out on 4/7/99 (Table 1) there was no significant decrease in mesophilic counts at 167.6 ppm or 327.5 ppm concentration. However, thermophiles showed a decrease of about 1 log unit at 167.6 ppm and 327.5 ppm concentration.

In the case of ammonium bisulfite (NH$_4$HSO$_3$) on experiments carried out on 5/12 and 7/28/99 the mesophiles decreased by greater than 1 to 1.5 log units and thermophiles by 1 to greater than 1.5 log units at 249.75 ppm NH$_4$HSO$_4$ concentration or 161.34 ppm SO$_2$ concentration. At 499.5 ppm NH$_4$HSO$_3$ or 322.67 ppm SO$_2$ concentration slight increases in kill levels of mesophiles (greater 1.5 to > 2 log units) and thermophiles (1.5 to >1.5 log units) was observed on 5/12 and 7/28/99 bench scale studies. In addition on 7/28/99 higher concentrations of NH$_4$HSO$_3$ of 749.25ppm (or 484.02 ppm SO$_2$) and 999.0 ppm (or 645.36 ppm SO$_2$) was evaluated. However, these higher levels of NH$_4$HSO$_3$ concentrations gave similar kill rates of >2 log units for mesophiles and greater than 1.5 log units for thermophiles which was very similar to the kill at 499.5 ppm NH$_4$HSO$_3$ (or 322.67 ppm SO$_2$). This showed that gaseous SO$_2$ at 363.8 ppm concentration had greater biocidal effect on mesophiles (3 log units) than NH$_4$HSO$_3$ at 999 ppm concentration or as 645 ppm SO$_2$.

**Biocide Factory Trial**
A biocide trial to evaluate the effectiveness of Kcide-800 (thiocarbamate) and Kcide-850 (glutaraldehyde) without the use of SO$_2$ was begun at Crookston factory on 4/29/99 and continued through 5/3/99. Samples were taken for microbial assessment (mesophiles, thermophiles, *Leuconostoc*, yeast and mold) daily through the trial period. Five locations in the factory were evaluated. (See Table 3 for detail)

In this study SO$_2$ was continuously added to cell 5 of the diffuser through the trial period. However, SO$_2$ was shut off in pulp press water (PPW) after the initial samples were taken and Kcide-800 was added instead. This resulted in drastic increases in mesophilic counts of greater than 2 log units (log 5.2 to log 7.56 cfu/g) in the pulp press water tank. A very significant increase in thermophilic counts of greater than 2.5 log units (from log 3.31 to log 5.89 cfu/g) in PPW tank was also observed. These changes were seen within 24 hours of addition of Kcide-800 to PPW and also resulted in a significant increase in L-lactic acid numbers from 299 to 1295 ppm at this location. However, *Leuconostoc* and yeast and mold counts did not show significant changes in numbers with the addition of Kcide-800. Also, due to the rapid increase in lactic acid on 4/30/99 both the addition of Kcide-850 (glutaraldehyde) and SO$_2$ was begun at the PPW location simultaneously though advised not to do so previously. Chemicals such as SO$_2$ and glutaraldehyde should never be added simultaneously due to the formation of the bisulfite addition product resulting in the unavailability of these biocides to act on microbes and have any biocidal effect. SO$_2$ was added to PPW continuously from 5/1/99 through 5/3/99. However, it took a couple of days before the mesophilic and thermophilic counts went back to the levels they were on 4/29/99 at the start of the experiment. The effect of the addition of Kcide-800 to PPW through a 24-hr period was seen in other regions with significant increases in thermophilic counts of greater than one log unit in locations such as the raw juice before heater, raw juice
surge tank, and diffuser cell 5 on 4/30/99. This also resulted in corresponding significant increases in lactic acid values from ~200 ppm to an increased range of approximately 972-1430 ppm in these regions. However, presently Kcide-800 is being applied directly on beets before and after the slicers and has shown promise. Further testing is in progress.

CONCLUSIONS

These studies have shown that:

1) Biocides such as Kcide-800 (thiocarbamate) and Kcide-850 (glutaraldehyde) are effective when microbial counts are at moderate levels of log 4.5 to 5.0 cfu/g for mesophiles and log 3.0 cfu/g for thermophiles. However, these biocides are ineffective or incapable of decreasing counts during high microbial loading such as log 7.0-8.0 for mesophiles (see Table 2). This is probably due to the maximum concentration of biocide permitted for use in the factory for Kcide-800 is 20 ppm and for Kcide-850 is 20-500 ppm. This is also probably one of the reasons that the Crookston factory got out of control within 24 hrs of use of Kcide-800 during trials carried out from 4/29 to 5/3/99. Another reason being that Kcide-850 and SO₂ was added simultaneously producing the bisulfite addition product and therefore the biocides were unavailable for control or decrease of microbes.
   • Further trials with addition of Kcide-800 directly on beets before and after the slicer are being carried out and have shown promise. Further testing is being carried out.

2) Tsunami-100 was found not to be as effective as Kcide-800 or Kcide-850. Also it showed significant biocidal effect only around 80 ppm on moderate levels of microbial populations.

3) Sulfur dioxide was found to be as effective or better on moderate levels of microbes at ~200 to 400 ppm concentration. Also, SO₂ has an advantage over other biocides in that it may be used at higher concentrations (above 400 ppm) to significantly decrease high microbial loading.

4) Sodium sulfite (Na₂SO₃) was found to have no effect on moderate levels of mesophiles at concentrations similar to SO₂ (~200 to 400 ppm). Also thermophiles were decreased only by about one log unit.

5) Ammonium bisulfite (ABS) at similar concentrations of SO₂ to gaseous SO₂ had much less biocidal effect on mesophiles and thermophiles at moderate microbial levels (see Table 1). This is similar to findings at British Sugar (Verbal communication – they did not have much luck with ABS). Also the cost of ammonium bisulfite is 3-5 times higher than gaseous SO₂.

ACKNOWLEDGEMENT

The authors wish to thank Jeff Moritz for the preparation of the raw juice without additives in the pilot plant which was used for bench studies. Our thanks are also due to Tom Samson (Production Superintendent) and Ken Hegge (Factory Chemist) for their help and support with the factory trials and Mary Johnson for typing the report.
### Biocides and Chemicals Used in Bench Studies

<table>
<thead>
<tr>
<th>Biocide / Chemical</th>
<th># of Studies</th>
<th>Concentrations Evaluated (ppm)</th>
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</thead>
<tbody>
<tr>
<td>Dithiocarbamate (Kcide - 800)</td>
<td>5</td>
<td>20, 40, 100, 400</td>
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<td>Kabo Chemicals</td>
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<td>23.52, 47.04, 117.6, 470.4</td>
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<td>Peroxy Acetic Acid / Hydrogen Peroxide (Tsunami-100)</td>
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<td>5, 80</td>
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<td>Eco Lab</td>
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<td>5.55, 88.8</td>
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<tr>
<td>Glutaraldehyde (50%) (Kcide-850)</td>
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<tr>
<td>Kabo Chemicals</td>
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<td>22.58, 282.25, 564.5</td>
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<tr>
<td>Sodium Sulfite (Na$_2$SO$_3$)</td>
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<td>200, 400</td>
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<td>167.6, 327.5</td>
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<tr>
<td>Ammonium Bisulfite (NH$_4$HSO$_3$) – 45% Solution</td>
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<td>200, 400, 600, 800</td>
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<td>249.75, 499.5, 749.25, 999.8</td>
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<tr>
<td>Sulfur Dioxide (SO$_2$)</td>
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<td>200, 400</td>
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<td>195.5, 363.8</td>
</tr>
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</table>
# BIocide Bench Studies

## Microbiological Results with KCide-800

<table>
<thead>
<tr>
<th>Biocide</th>
<th>Trial Type &amp; Date</th>
<th>Types of Microbes</th>
<th>Raw Juice Initial Log Count (cfu/g)</th>
<th>Control Log Count (cfu/g)</th>
<th>Microbial Count in Different Concentrations of Biocide Used (ppm)</th>
</tr>
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<tbody>
<tr>
<td>Kcide 800</td>
<td>Trial 1 (1 ¾ Days)</td>
<td>Mesophiles</td>
<td>4.58</td>
<td>6.68</td>
<td>23.52 47.04 117.6 470.4</td>
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<td>Thermophiles</td>
<td>1.78</td>
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<tr>
<td>Kcide 800</td>
<td>Trial 2 (1 ¾ Days)</td>
<td>Mesophiles</td>
<td>4.83</td>
<td>6.92</td>
<td>3.89 (avg) - - - -</td>
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<tr>
<td></td>
<td>4-7-99</td>
<td>Thermophiles</td>
<td>3.07</td>
<td>3.33</td>
<td>2.02 (avg) - - - -</td>
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<td>Kcide 800</td>
<td>Trial 3* (2 ¾ Days)</td>
<td>Mesophiles</td>
<td>8.03</td>
<td>7.98</td>
<td>8.46 8.61</td>
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<td>3-15-99</td>
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<td>3.04</td>
<td>3.23 3.34</td>
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<td>Kcide 800</td>
<td>Trial 4* (3 ¾ Days)</td>
<td>Mesophiles</td>
<td>8.53</td>
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<td>8.24 8.26</td>
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<td>3-16-99</td>
<td>Thermophiles</td>
<td>2.95</td>
<td>-</td>
<td>2.15 2.15</td>
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<tr>
<td>Kcide 800</td>
<td>Trial 5 (2 ¾ Days)</td>
<td>Mesophiles</td>
<td>7.82</td>
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<td>3-22-99</td>
<td>Thermophiles</td>
<td>3.51</td>
<td>2.18</td>
<td>- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -</td>
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</tbody>
</table>

* In these 2 trials (#3 & #4) the samples were incubated for 30 minutes at 25°C after addition of the biocide Kcide-800. (In all other trials - #s 1, 2, & 5, the samples were incubated for 5 hrs at 40°C.)

**NOTE:** The days in parentheses in Column 2 refer to the time the frozen raw juice was thawed out for use in trials.
## BIOCIDE BENCH STUDIES
### MICROBIOLOGICAL RESULTS WITH TSUNAMI-100

<table>
<thead>
<tr>
<th>Biocide</th>
<th>Trial Type &amp; Date</th>
<th>Types of Microbes</th>
<th>Raw Juice Initial Log Count (cfu/g)</th>
<th>Control Log Count (cfu/g)</th>
<th>Microbial Count in Different Concentrations of Biocide Used (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsunami-100</td>
<td>Trial 1 (1 ¾ days)</td>
<td>Mesophiles</td>
<td>4.97</td>
<td>7.37</td>
<td>7.38 6.24</td>
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<tr>
<td>Peroxy acetic acid &amp; hydrogen peroxide</td>
<td>3-24-99</td>
<td>Thermophiles</td>
<td>3.86</td>
<td>3.87</td>
<td>3.77 2.30</td>
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<tr>
<td>Tsunami-100</td>
<td>Trial 2 (1 ¾ days)</td>
<td>Mesophiles</td>
<td>4.94</td>
<td>8.32</td>
<td>8.40 (avg) 8.26 (avg)</td>
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<td>6-9-99</td>
<td>Thermophiles</td>
<td>2.20</td>
<td>3.18</td>
<td>3.36 (avg) 2.66 (avg)</td>
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<tr>
<td>Tsunami-100</td>
<td>Trial 3* (4 ¾ days)</td>
<td>Mesophiles</td>
<td>8.46</td>
<td>-</td>
<td>8.58 8.49</td>
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<td>3-17-99</td>
<td>Thermophiles</td>
<td>3.51</td>
<td>-</td>
<td>3.08 2.30</td>
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</table>

* In Trial #3 with Tsunami-100 the sample was incubated for 30 min. at 25°C (while in all other trials the samples were incubated for 5 hrs at 40°C).

**NOTE:** The days in parentheses in Column 2 refer to the time the frozen raw juice was thawed out for use in trials.
## BIOCIDÉ BENCH STUDIES
### MICROBIOLOGICAL RESULTS WITH KClIDE-850

<table>
<thead>
<tr>
<th>Biocide</th>
<th>Trial Type &amp; Date</th>
<th>Types of Microbes</th>
<th>Raw Juice Initial Log Count (cfu/g)</th>
<th>Control Log Count (cfu/g)</th>
<th>Microbial Count in Different Concentrations of Biocide Used (ppm)</th>
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</thead>
<tbody>
<tr>
<td>Kcide-850</td>
<td>Trial 1 (1 ¾ days) 5-12-99</td>
<td>Mesophiles</td>
<td>5.21</td>
<td>7.78</td>
<td>22.58: 3.99 (avg) 282.25: 3.96 (avg) 564.5: 3.97 (avg)</td>
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<td>Thermophiles</td>
<td>1.60</td>
<td>3.52</td>
<td>22.58: 2.04 (avg) 282.25: 1.78 (avg) 564.5: 1.90 (avg)</td>
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<td>Mesophiles</td>
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<td>Thermophiles</td>
<td>2.20</td>
<td>3.18</td>
<td>22.58: 2.08 (avg) 282.25: - (avg) 564.5: - (avg)</td>
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<tr>
<td>Kcide-850</td>
<td>Trial 3 (1 day) 4-28-99</td>
<td>Mesophiles</td>
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<td>4.37</td>
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<td>Thermophiles</td>
<td>2.18</td>
<td>2.04</td>
<td>22.58: 1.95 (avg) 282.25: 2.04 (avg) 564.5: 1.95 (avg)</td>
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</table>

**NOTE:**

1. The days in parentheses in Column 2 refer to the time the frozen raw juice was thawed out for use in trials.
2. In all above Kcide-850 trials the samples were incubated for 5 hrs at 40°C.
### BIocide Bench Studies

**Microbiological Results with Sulfur Dioxide and Sodium Sulfite**

<table>
<thead>
<tr>
<th>Chemical Tested</th>
<th>Trial Type &amp; Date</th>
<th>Types of Microbes</th>
<th>Raw Juice Initial Log Count (cfu/g)</th>
<th>Control Log Count (cfu/g)</th>
<th>Microbial Counts in Different Concentrations of Chemicals Used (ppm)</th>
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<tbody>
<tr>
<td>Sulfur Dioxide (SO₂) Sparged</td>
<td>Trial 1 (1 ¾ days) 4-7-99</td>
<td>Mesophiles</td>
<td>4.83</td>
<td>6.92</td>
<td>195.5 363.8</td>
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<td>Thermophiles</td>
<td>3.07</td>
<td>3.33</td>
<td>4.15 3.92</td>
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<th>Types of Microbes</th>
<th>Raw Juice Initial Log Count (cfu/g)</th>
<th>Control Log Count (cfu/g)</th>
<th>Microbial Counts in Different Concentrations of Chemicals Used (ppm)</th>
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<tr>
<td>Sodium Sulfite (Na₂SO₃)</td>
<td>Trial 1 (1 ¾ days) 4-7-99</td>
<td>Mesophiles</td>
<td>4.83</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>2.23 2.28</td>
</tr>
</tbody>
</table>

**Note:**

1. The days in parentheses in Column 2 refer to the time the frozen raw juice was thawed out for use in trials.
2. In above trials, the samples were incubated for 5 hrs at 40°C.
## BIOCIDE BENCH STUDIES
### MICROBIOLOGICAL RESULTS WITH AMMONIUM BISULFITE

<table>
<thead>
<tr>
<th>Chemicals Tested</th>
<th>Trial Type &amp; Date</th>
<th>Types of Microbes</th>
<th>Raw Juice Initial Log Count (cfu/g)</th>
<th>Control Log Count (cfu/g)</th>
<th>Microbial Count in Different Concentrations of Chemicals Used (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Bisulfite (NH₄HSO₃)</td>
<td>Trial 1 (1 ¾ Days) 5-12-99</td>
<td>Mesophiles</td>
<td>5.21</td>
<td>7.78</td>
<td>6.56 (avg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thermophiles</td>
<td>1.60</td>
<td>3.52</td>
<td>2.11 (avg)</td>
</tr>
<tr>
<td>Ammonium Bisulfite (NH₄HSO₃)</td>
<td>Trial 2 (1 ¾ Days) 7-28-99</td>
<td>Mesophiles</td>
<td>4.94</td>
<td>6.91</td>
<td>5.41 (avg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thermophiles</td>
<td>1.70</td>
<td>3.68</td>
<td>1.91 (avg)</td>
</tr>
</tbody>
</table>

**NOTE:**

1. The days in parentheses in Column 2 refer to the time the frozen raw juice was thawed out for use in trials.
2. In all above NH₄HSO₃ trials, the samples were incubated for 5 hrs at 40°C.
MICROBIAL COUNTS IN PRESS PULP WATER TANK DURING BIOCIDE TRIAL AT ACS FACTORY

Date (Biocide)

- 4-29-99 (Initial)
- 4-30-99 (K-Cide 800)
- 5-1-99 (SO2)
- 5-2-99 (SO2)
- 5-3-99 (SO2)

Log Count

- 8.00
- 7.00
- 6.00
- 5.00
- 4.00
- 3.00
- 2.00
- 1.00
- 0.00

Types of Microbes
- Mesophiles
- Thermophiles
- Leuconostoc
- Yeast
- Mold
MICROBIAL COUNTS IN RAW JUICE BEFORE HEATER DURING BIOCIDE TRIAL AT ACS FACTORY

DATE (BIOCIDE)

LOG COUNT

Mesophiles
Thermophiles
Leuconostoc
Yeast
Mold

1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00

4-29-99 (Initial)  4-30-99 (K-Cide 800)  5-1-99 (SO2)  5-2-99 (SO2)  5-3-99 (SO2)
MICROBIAL COUNTS IN RAW JUICE SURGE TANK DURING BIOCIDAL TRIAL AT ACS FACTORY

- Mesophiles
- Thermophiles
- Leuconostoc
- Yeast
- Mold

DATE (BIOCIDE)

4-29-99 (Initial)  4-30-99 (K-Cide 800)  5-1-99 (SO2)  5-2-99 (SO2)  5-3-99 (SO2)

LOG COUNT

0.00  1.00  2.00  3.00  4.00  5.00  6.00  7.00  8.00
MICROBIAL COUNTS IN DIFFUSER CELL 5 DURING BIOCIDAL TRIAL AT ACS FACTORY

**Graph Description:**
- **Axes:**
  - Vertical: Log Count
  - Horizontal: Date of Biocide Application
- **Legend:**
  - Mesophiles
  - Thermophiles
  - Leuconostoc
  - Yeast
  - Mold
- **Dates and Biocides:**
  - 4-29-99 (Initial)
  - 4-30-99 (K-Cide 800)
  - 5-1-99 (SO2)
  - 5-2-99 (SO2)
  - 5-3-99 (SO2)
MICROBIAL COUNTS IN DIFFUSER CELL 1 DURING BIocide TRIAL AT ACS FACTORY

LOG COUNT

DATE (BIocide)

- Mesophiles
- Thermophiles
- Leuconostoc
- Yeast
- Mold