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Reducing the Amount of Microbiological Growth Using Ultrasonic Treatment

Mark Gill
Western Michigan University

Reducing the Amount of Microbiological Growth Using Ultrasonic Treatment

by

Mark Gill

Advisor: Bill Forester

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ABSTRACT

This thesis explored the possibility of using ultrasonic treatment to reduce the amount of microbiological growth in a sample of contaminated mill wastewater. It was found that ultrasonic treatment reduces microbiological growth through the mechanism of cavitation. percent inhibition increases with both time of treatment and applied power. The most economical conditions were high power and a short treatment time.

The increase in cell volume had an inverse effect on the percent inhibition. The increase in consistency of the treated solution also had an inverse effect on the percent inhibition and 6% was the maximum treatable consistency.

Ultrasonic treatment was compared to three popular biocides at a 90% inhibition level. The biocides used were Methylene Bis(Thiocyanate), Isothiazolin, and a Thiadiazine type. Dosages to achieve 90% inhibition were 154 ppm., 161 ppm., and 127 ppm. respectively. The 90% inhibition level was reached with 0.0158 kW hrs. of energy, using ultrasonic treatment. This energy was scaled up to 8.020/ HP day/ton.

Further studies are recommended in the areas of cell geometry, determination of the maximum power efficiency, and the developement of a dynamic model to test ultrasonic treatment in a situation oriented more towards the paper mill enviroment.

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INTRODUCTION

The goal of this thesis project is to determine if ultrasonic treatment can kill the types of microbiological growth that are found in the paper machine system. Samples of contaminated whitewater will be subjected to various power levels of ultrasonic treatment for a variety of time intervals. The same whitewater will be treated with three popular biocides to determine the amount needed to obtain a 90% inhibition level of microbiological growth.

The results for all ultrasonic treatments will be compared to determine if the ultrasonic treatment had an effect on reducing the total amount of biological growth and which power level was most economical. If there is any reduction of the total count due to ultrasonic treatment, a comparison with the biocides will be made at 90% inhibition. The power consumption of the ultrasonic treatment will be compared to the amount of biocide needed to achieve 90% inhibition. The determination of the trends concerning consistency and cell volume of the treated samples will also be discussed.

THEORETICAL DISCUSSION

Background:

Microbiological growth can cause major problems in many areas. Aerobes, which require oxygen to exist, are generally slime formers. The production of slime can result in strings which will create streaks in the sheet or entire deposits can break loose and flow through the system. These deposits can cause large holes in the sheet or even create a break in the web (10).

A turbucle is another type of deposit that is formed in the papermaking system. This is a hard circular deposit that forms on the inside of pipes. The aerobes trapped underneath the turbucle use up all the air and die. Faculative anaerobes, which do not breathe air, multiply rapidly and produce H_2S. The presence of H_2S and oxygen produces H_2SO_4 which corrodes the pipe and produces a small green dot on the outside of the pipe. This dot will soon become a leak. All anaerobes produce H_2S and can lead to corrosion problems throughout the machine (2,3,10).

There are many types of chemicals that are manufactured to reduce the amount of microbiological growth in the papermachine system. These chemicals are called biocides and are specifically designed to eliminate biological growth. There are some draw-backs associated with the use of biocides. First, biocides are fairly expensive so as little as possible must be used. Secondly, some of the biocides,

mostly the chlorine type compounds, can cause corrosion and machine wear (4).

If a method of reducing the total microbiological count of a system could be devised, without producing machine wear or effluent clean-up problems, it would be an extreme benefit to the paper industry. Some newer chemicals, such as iso-thiazolin, can be neutralized to reduce the effluent load. My thesis will explore the possibility of using ultrasonic treatment to reduce the total amount of biological growth. Ultrasonic treatment would produce no machine wear or any increase in the effluent load.

Ultrasonic Treatment:

An ultrasonic wave traveling through a medium consists of alternating compressions and rarefactions. The rarefactions are the regions of low pressure in a medium traversed by compressional waves. If the wave is applied to a liquid and is strong enough, countless microscopic bubbles are formed and then collapsed. This phenomenon is known as cavitation. Cavitation is imparitive to the reduction of microbiologic growth. If the energy in the wave is sufficiently intense, the bubbles collapse with great force, producing local pressure changes of thousands of atmospheres. The release of mechanical energy is so intense that it is able to totally destroy cell walls and tear particles apart (5).

To produce ultrasonic waves of high intensity I will be using the W185 Sonifier Cell Disruptor. The Sonifier Cell Disruptor consists of a transistorized power supply , a converter, and a disruptor horn (see appendix #1). The converter uses a lead zirconate titanate ceramic as the active piezoelectric element. The converter has a fixed frequency and operates at 20 kHz. The horn is used as a mechanical transformer. It is used to achieve the proper force amplitude ratio between the converter and the liquid (5). There are a variety of horns available but the standard disruptor horn is used for disintigration of cells.

Temperature:

When energy, such as ultrasonic waves, is applied to a liquid, the temperature of the liquid is increased. If the temperature of the treated solutions is raised above 140°F the solution could be sterilized by heat (1,6). This would be included in the percent inhibition of the treatment and a false value would result. To eliminate the possibility of thermal sterilization the samples will be cooled while being treated. During the ultrasonic treatment the temperature of the solutions will be monitored to assure that they do not reach the sterilization temperature. After the samples are treated they will be cooled to inhibit any further growth activity. Samples of whitewater will be refrigerated until they are used to retard any further growth.

Biocides:

The ultrasonic treatment will be compared to three popular biocides. These biocides were obtained from Betz PaperChem Inc. The biocides used were:

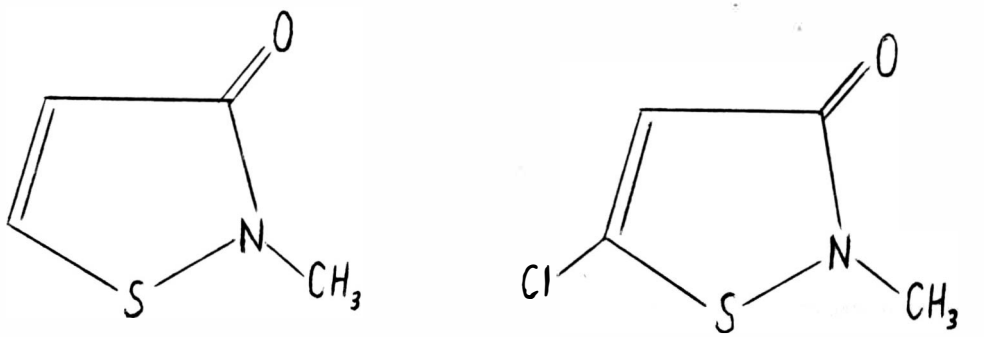
A) 2-methyl-4-isothiazolin-3-one
with 5-chloro-2-methyl-4-isothiazolin-3-one
commonly called Isothiazolin

B) Methylene Bis(Thiocyanate) commonly called MBT

C) Tetrahydro-3,5-Dimethyl-2H-1,3,5-Thiadiazine-2-Thione
commonly called Thiadiazine

The structures of these biocides are as shown below in Figure #1 (7):

A.



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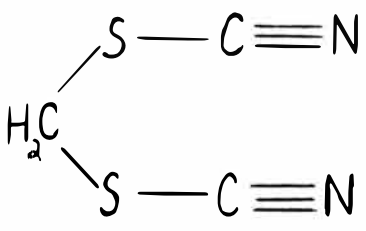

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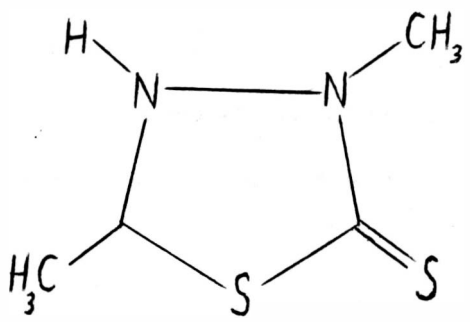


Figure #1: Structures of biocides.

These biocides destroy the microbiological growth by interupting the enzyme production in the cell. When the biocides come in contact with the cell the rings open and the thione groups interfere with the thione groups present in the cell. This interference disrupts the normal enzyme production of the cell and desroys the cell. The MBT biocide also interupts the enzyme production through the cyanide groups (7).

EXPERIMENTAL PROCEDURE

Materials:

The materials needed to perform the total counts and toxic evaluations for this experiment consisted of conventional microbiological plating materials. These materials were:

1. Petri dishes (100 mm. * 20 mm.)
2. Disposable pipets:
   A. 1/10 ml. incremented for biocide measurements.
   B. 0.5, 1.0, and 1.1 ml. incremented for serial dilutions.
3. Tryptone Glucose Extract Agar (T-agar).
4. Sterile 99 ml. water blanks for serial dilutions.
5. 50 ml. graduated cylinder for contaminated water measurement.

These materials were obtained from the WMU paper department stockroom and Bill Forester.

Chemicals:

The only chemicals needed for this experiment were the biocides. These chemicals were obtained from Betz Paperchem, Jacksonville Florida. The three biocides recieved were:

1. RX-32A Methylene Bis(Thiocyanate).
2. RX-68 2-methyl-4-Isothiazolin-3-one and 5-chloro-2-methyl-4-Isothiazolin-3-one.
3. RX-28 Tetrahydro-3,5-dimethyl-2H-1,3,5-Thiadiazine-2-thione.

Equipment:

The equipment needed for this experiment was obtained by individual case. The ultrasonic unit, Sonifier Cell Disruptor, was obtained through Dr. Stephen Friedman of the WMU Biomedical Sciences department. This unit was used to treat the samples of contaminated water.

An incubator was needed to store the petri dishes while the colonies were growing. A walk-in incubator was used in the microbiology lab. Permission was obtained from Ms. Vivian Locke. She is the senior lab technician in charge of the lab. The incubator is kept at a constant temperature of 37°C.

An oven for holding the cooked agar was also obtained from Ms. Locke. This oven was kept at 55° C. The T-agar is in a solid phase at room temperature and must be heated to obtain the liquid phase. The oven is used to keep the T-agar hot enough to remain in the liquid phase, but not hot enough to sterilize the plated samples.

A pressure cooker for making T-agar and also sterilizing the T-agar was used in the microbiology lab. Production of T-agar was supervised by Ms. Locke. All ingredients for the T-agar were obtained from the lab. The T-agar made in the lab was consistent with the T-agar obtained from the paper department (8).

Procedure:

To begin this thesis, a sample of whitewater was obtained from the James River Boardmill. The water sample was obtained from the open canal which routes the waste whitewater from the machines to the clarifiers. Using this sample, a control was grown in a T-agar solution to determine the amount of microbiological growth present, the total count. This count was determined by the serial dilution method shown in appendix #2.

The three biocides were then tested on the contaminated whitewater sample to determine the percent inhibition at 10 ppm., 50 ppm , 100 ppm., and 150 ppm. A linear regression was then performed to determine the parts per million (ppm.) of biocide needed to obtain 90% inhibition. Using these biocides a dilution type toxicant evaluation was performed (see appendix #3). The treatment time for the biocides was fifteen minutes. This test will show which biocides are the most effective and at what concentrations an acceptable microbiological growth reduction of 90% can be achieved.

Samples of the whitewater were also subjected to ultrasonic treatment. The intensity, controlled by the output of the power supply, and the time of treatment was varied to determine the most adventagious conditions at which the ultrasonic treatment should be applied. The ultrasonic unit was run at power levels 3, 5, and 7 on a unitless scale. The power output, in watts, was recorded for each run so an energy calculation may be performed. The samples were treated for 1, 3, 5, 7, and 9 minute intervals. The sample size was 100 ml.

The temperature of the whitewater samples was controlled by placing the samples in an ice bath. Other conditions, such as consistency and cell volume remained constant. The samples were mixed every fifteen seconds to assure thorough treatment through out the sample. The samples were then plated to determine the amount of microbiological growth still present. Controls were also plated and a determination of percent inhibition was calculated.

After determining the most efficient power level for the inhibition of microorganisms, (level 7), the samples were treated at various cell volumes. Samples of 50 ml., 100 ml., 150 ml., 200 ml., and 250 ml. were treated at a constant time interval and consistency. The treatment time was 5 minutes and the consistency was 0.014%. The solutions were then plated with controls to determine the percent inhibition.

A plot of cell volume vs. percent inhibition will show the trend that increasing the cell volume has on percent inhibition.

Also at the most efficient power level, samples of varying consistency were treated at a constant time interval and cell volume (100ml.). Samples of 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, and 6.0% consistency were treated. The sample solutions and controls were then plated and the percent inhibition for each run was calculated. A plot of percent consistency vs. percent inhibition will show the trend that increasing consistency has on percent inhibition using a constant cell volume.

All runs were performed in duplicate. Data for individual runs is shown in appendix #4.

RESULTS

The following results were obtained from data compiled during this experiment.

Table #1: Percent Inhibition for Power Levels at Various Times.

Time (m)	Level 3	Level 5	Level 7
1	15.0	17.9	23.1
3	28.0	39.9	46.0
5	33.0	42.8	52.6
7	42.0	56.6	71.9
9	53.0	75.4	88.0

* All Values in Percent (%).

Table #2: Average Power Output for Various Levels.

Level	Power Output (Watts)
3	80.2
5	89.1
7	101.8

Table #3: Power and Energy Output for Levels at Various Times.

Time (m)	Level 3		Level 5		Level 7	
	Power	Energy	Power	Energy	Power	Energy
1	81.5	0.0014	87.0	0.0015	100.0	0.0017
3	82.0	0.0041	87.0	0.0044	99.0	0.0050
5	78.5	0.0065	90.0	0.0075	104.0	0.0087
7	80.0	0.0093	91.0	0.0106	102.0	0.0119
9	79.0	0.0119	90.5	0.0136	104.0	0.0156

Energy = kW hrs

Power = Watts

Table #4: Percent Inhibition for Biocides at Various Dosages.

Dosage (ppm)	MBT	Isothiazolin	Thiadiazine
10	67.7	59.9	66.9
50	74.3	65.3	77.3
100	81.4	77.9	85.9
150	89.4	87.7	93.2

* All Values in Percent (%).

Table #5: Comparison of Biocides to Ultrasonic Treatment at 90% Inhibition.

Biocides		Ultrasonic Treatment		
Type	ppm needed	Level	Time (m)	Energy (kW hrs)
MBT	154	3	17.4	0.0233
Isothiazolin	161	5	11.6	0.0172
Thiadiazine	127	7	9.3	0.0158

* All Values Calculated From Linear Regression Data.

Table #6: Linear Regression Data for Ultrasonic Treatment and Biocides.

Treatment	Equation of Line
Ultrasonic Level 3	$y = (4.50)x + (11.70)$
Ultrasonic Level 5	$y = (6.59)x + (13.60)$
Ultrasonic Level 7	$y = (7.79)x + (17.40)$
MBT Biocide	$y = (0.15)x + (66.30)$
Isothiazolin Biocide	$y = (0.21)x + (56.80)$
Thiadiazine Biocide	$y = (0.18)x + (66.49)$

Table #7: Percent Inhibition for Ultrasonic Treatment at Various Cell Volumes.

Cell Volume (ml.)	% Inhibition
50	66.2
100	50.6
150	43.2
200	36.5
250	18.1

* All samples were treated for 5 minutes at power level 7.

Table #8: Percent Inhibition for Ultrasonic Treatment at Various Consistencies.

Consistency (%)	% Inhibition
0.014	59.4
1.000	42.9
2.000	34.0
3.000	21.1
4.000	14.0
5.000	8.7
6.000	2.1

* All samples were 100 ml. and treated for 5 minutes at power level 7.

Table #9: Conversion of Energy Output.

Most efficient ultrasonic run = 0.0158 kW hrs./100 ml.

0.0158 kW hrs./100 ml. * 1.341 = 0.0212 HPhrs./100 ml.

0.0212 HP hrs./100 ml. * (1 day/24 hrs) = 0.0008833 HP day/100 ml.

0.0008833 HP day/100 ml. * (1 ml/1 g) * (454 g/ 1b) = 0.0040101 HPday/1b

0.0040101 HP day/1b. * (2000 1b/ 1 ton) = 8.0207 HPday/ton

Table #10: Statistical Analysis of Ultrasonic Treatment Runs.

Source	SS	df	MS	Fo	F
A (Time)	9745.518	4	2436.380	38.63	4.89
B (Power)	2441.882	2	1220.941	19.36	6.36
AB (Error)	464.078	8	58.010	0.92	4.00
Error	946.085	15	63.072		
Total	13597.563	29			

* F values from F(0.01,r1,r2) table showing a 99% Confidence Interval (9).

Figure #2:

Effect of Time on Percent Inhibition for Various Power Levels

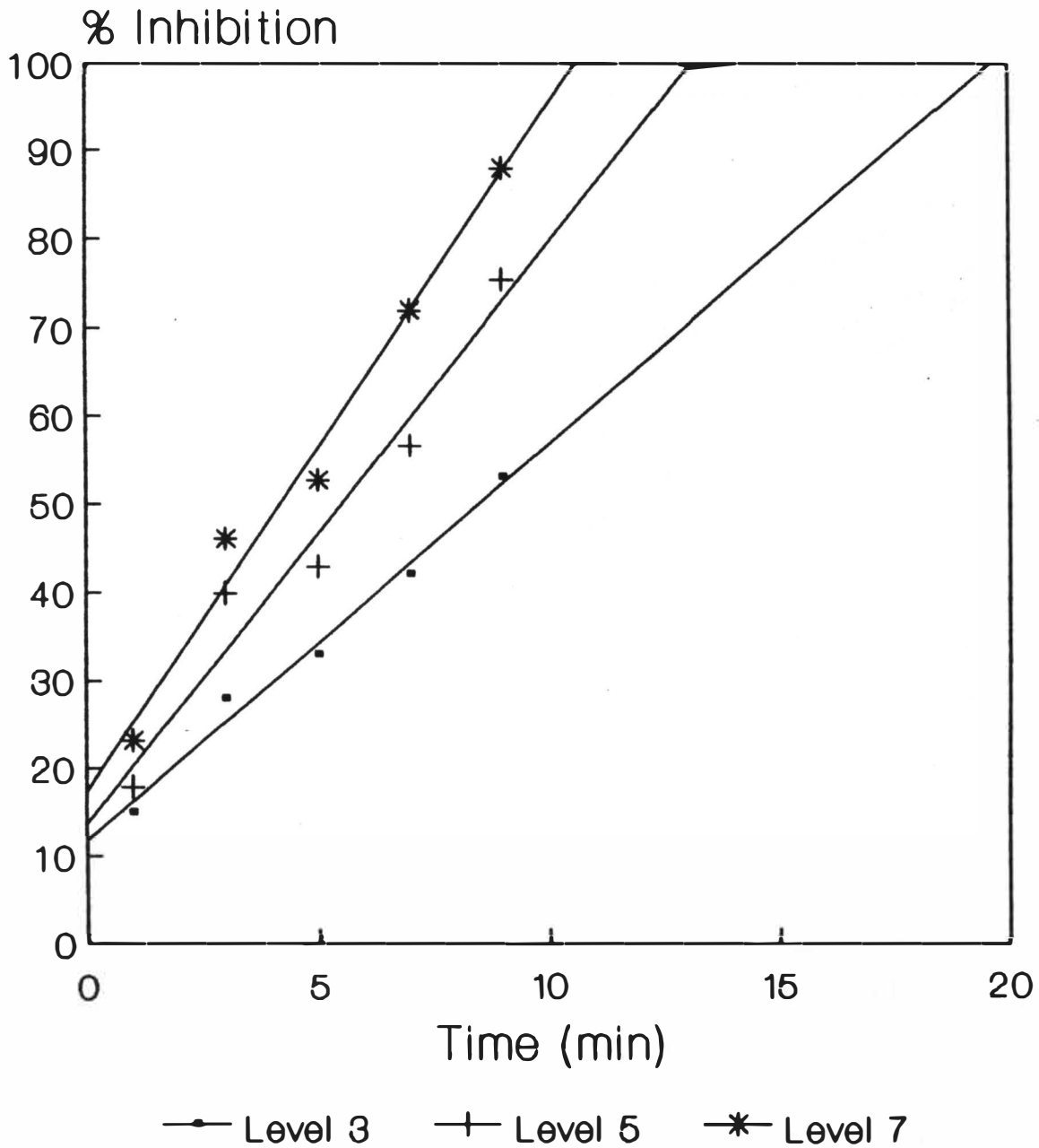


Figure #3:

Effect of Biocides on Percent Inhibition

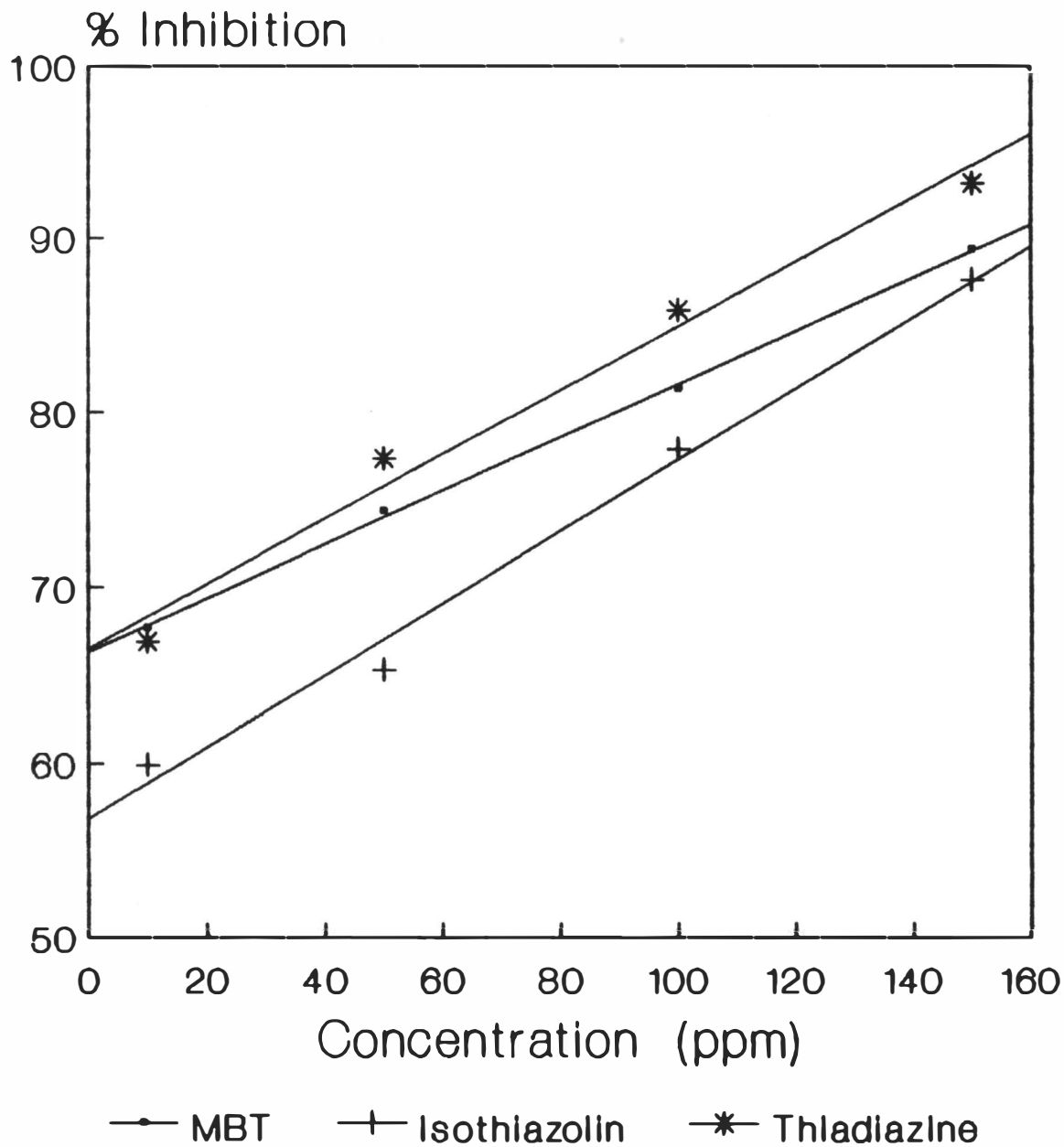


Figure #4:

Effect of Cell Volume on Percent Inhibition

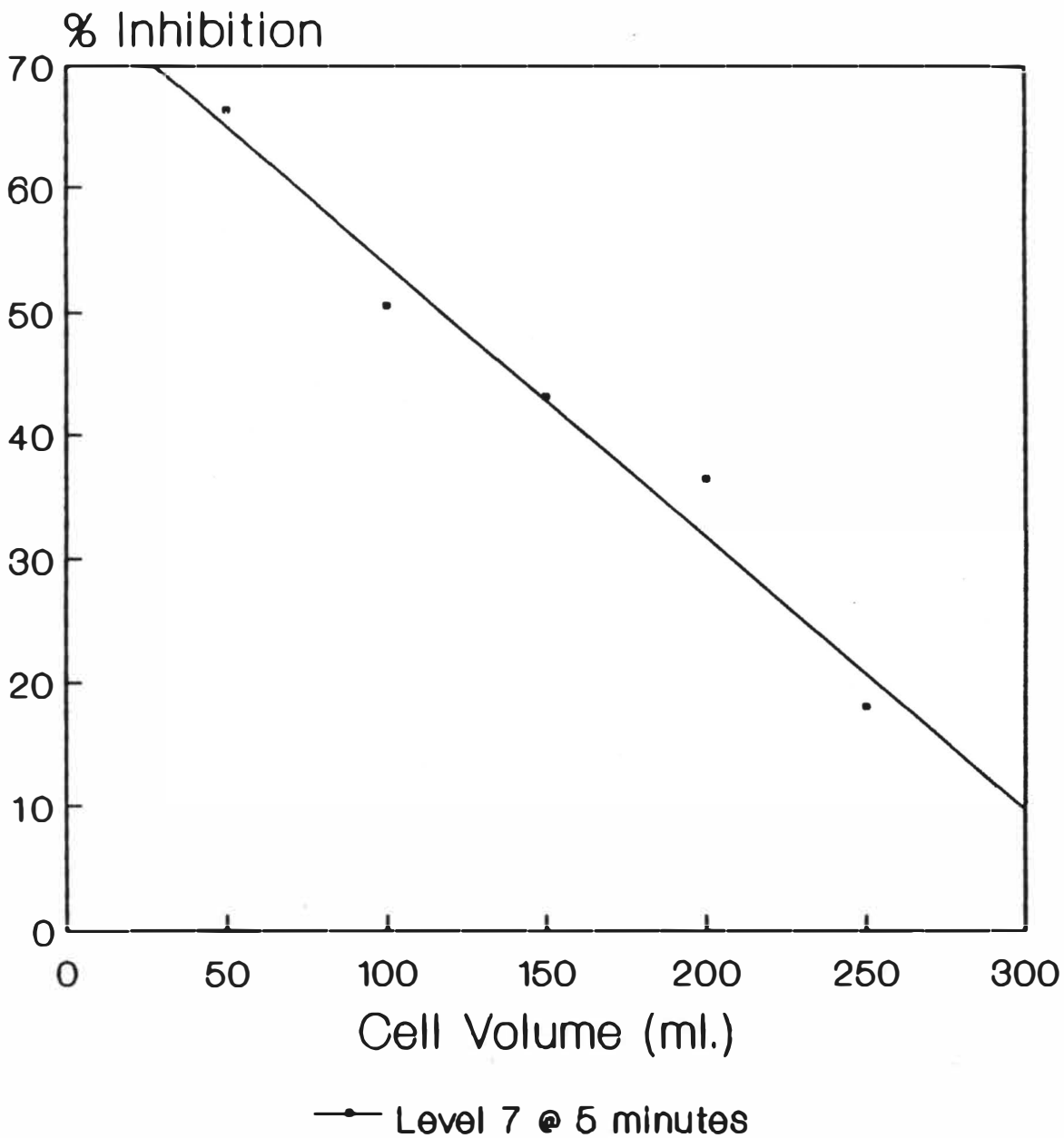
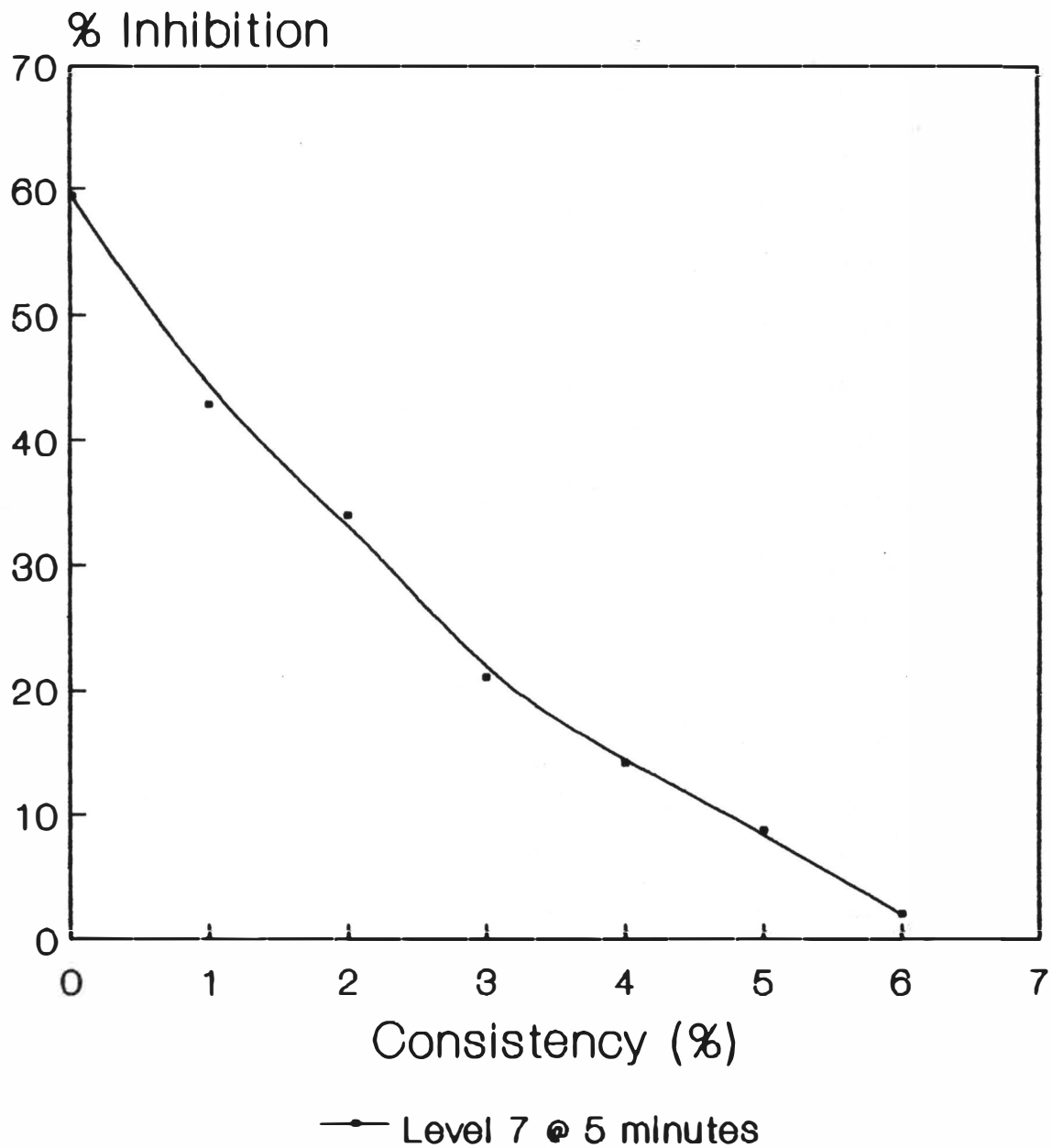


Figure #5:

Effect of Consistency on Percent Inhibition



DISCUSSION OF RESULTS

The effect of ultrasonic treatment on microbiological growth is shown in table #1 and in figure #2. Table #1 shows the percent inhibition for the various ultrasonic treatment runs. Table #1 also shows that, as the treatment time or the power level is increased, the percent inhibition is also increased. Figure #2 is a plot of percent inhibition verse time for various power levels. The plotted lines are the result of a linear regression performed on the data. These lines represent the inhibition rates for the various power levels in amount of microbiological growth reduced per unit time. Figure #2 also shows that power level 7 had the highest inhibition rate. This is indicated by the fact that the level 7 line has the highest slope. As shown by these trends, we can see that ultrasonic treatment had a definite effect on the reduction of microbiological growth.

Table #2 shows the average power output, in watts, for each level. Table #3 shows the actual power output for each run and the energy applied to the solution in kilowatt hours. Table #3 also shows that as the time of treatment is increased the amount of energy required is also increased.

Table #4 and figure #3 show the effect that the three biocides had on the amount of microbiological growth. Figure #3 is a plot of percent inhibition verse concentration of the biocide, in parts per million. The lines represent the trends and are calculated from a linear regression of the data. The linear regression for the three biocides and the

various power levels of ultrasonic treatment are shown in table #6. Table #4 shows the percent inhibition for the various dosages of each biocide.

Table #5 shows a comparison of the biocides to the ultrasonic treatment at 90% inhibition. The thiadiazine biocide was the most effective biocide at reducing the microbiological growth. This biocide needed 127 ppm. to achieve a 90% inhibition. The MBT and the isothiazolin biocides were quite comparible and needed 154 ppm. and 161 ppm. respectively. Table #5 also shows that as the power of the ultrasonic treatment is increased the treatment time is decreased. The most interesting factor is that the lowest energy is obtained from the highest power level. This shows that the most economical application of ultrasonic treatment is achieved with a high power dosage for a short period of time. Table #9 shows that the most economical run resulted in an energy consumption of 8.0207 HP day/ton. These values were calculated from the linear regression lines shown in table #6.

Table #7 and figure #4 show the effect of varying the treatment cell volume. Table #7 shows the percent inhibition for various cell volumes treated for 5 minutes at power level 7. As the cell volume is increased the percent inhibition decreases. This trend is shown in figure #4 which is a plot of percent inhibition verse cell volume in milliliters. From this data we can conclude that the percent inhibition is inversely proportional to the cell volume.

The effect of sample consistency on percent inhibition is shown in table #8 and in figure #5. Samples of 100 ml. were treated for 5 minutes at power level 7. Table #8 shows the percent inhibition for all runs. As the consistency is increased the percent inhibition decreases. This trend shows that percent inhibition is inversely proportional to consistency of the treated solution. Figure #5 is a plot of percent inhibition verse percent consistency which shows this trend. The plot ends at 6.0% consistency were there was no visible cavitation in the solution. It is believed that 6.0% consistency is the limit for ultrasonic treatment.

Due to the large variations incurred when plating microbiological colonies, a statistical analysis was necessary to prove that the observed trends were significant. Table #10 shows the results of this analysis. Using the sum of squares (SS), the degrees of freedom (df), and the mean squared values (MS) a calculated Fo value was determined. This value was compared to the F values from a 99% confidence interval table. The table shows that the variation in treatment time was significant being $Fo > F$. The variation of the power levels was also significant being $Fo > F$. The interaction between treatment time and power level (AB) is not significant being $Fo < F$. The lack of interaction is also shown in figure #2 being the trend lines are relatively parallel. If there was interaction between treatment time and the power levels then the trend lines would cross over one another.

CONCLUSIONS

In this thesis experiment it was found that ultrasonic treatment did have a positive effect on the reduction of microbiological growth. The ultrasonic treatment works through a mechanism of cavitation in the liquid solution to destroy the microbiological cells.

The percent inhibition increased with both time of treatment and applied power. The most economical conditions were high power and low treatment time.

The increase in cell volume had an inverse effect on the percent inhibition. As the volume of the treatment cell was increased the percent inhibition decreased.

The increase in consistency was also inversely proportional to percent inhibition. As the consistency of the treated solution was increased the percent inhibition decreased. The maximum consistency which was treatable was 6.0%. At this consistency there was no visible cavitation.

The ultrasonic treatment compared to the biocide dosages of approximately 150 parts per million when using the most popular market biocides.

RECOMMENDATIONS

To further this thesis study I recommend the following work.

This experiment was a static model, therefore a scale-up to a per ton basis was not applicable. Also a scale-up of this nature implies that this data would work in the mill situation, which is a dynamic system. If an ultrasonic treatment unit could be fitted to a pipe of flowing stock, a dynamic situation could be simulated. The effects of flow rate, consistency, and cross sectional area of the pipe could be explored. This data would be one step closer to the actual mill situation.

The next logical step would be to use a number of ultrasonic units in series on a pipe. This would give longer treatment times at any flow rate. This experiment would determine if there is any interaction between ultrasonic units, such as a cancelling out of waves due to phase problems or an increase in in applied energy due to a compounding effect.

Obtaining an ultrasonic unit that has an output greater than 105 watts would give the possibility of finding out if there is a maximum power level on the basis of energy efficiency. The unit I used had a 105 watt maximum which limited my research. Also a study of cell geometry would be interesting. This would correlate to the type of pipe inwhich the ultrasonic tip should be placed.

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Appendix #1: Diagram of the Ultrasonic Treatment Unit.

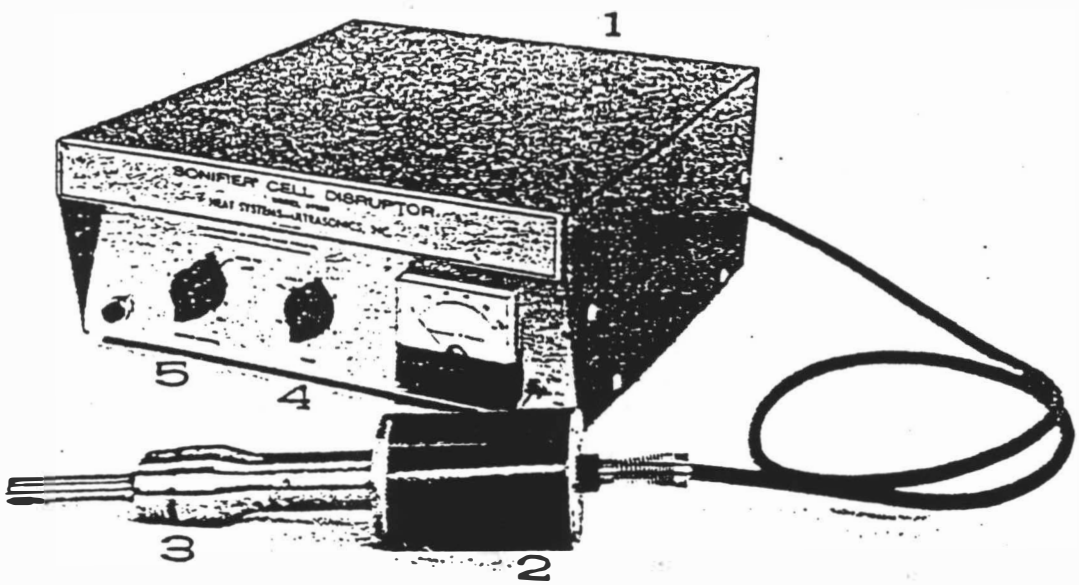
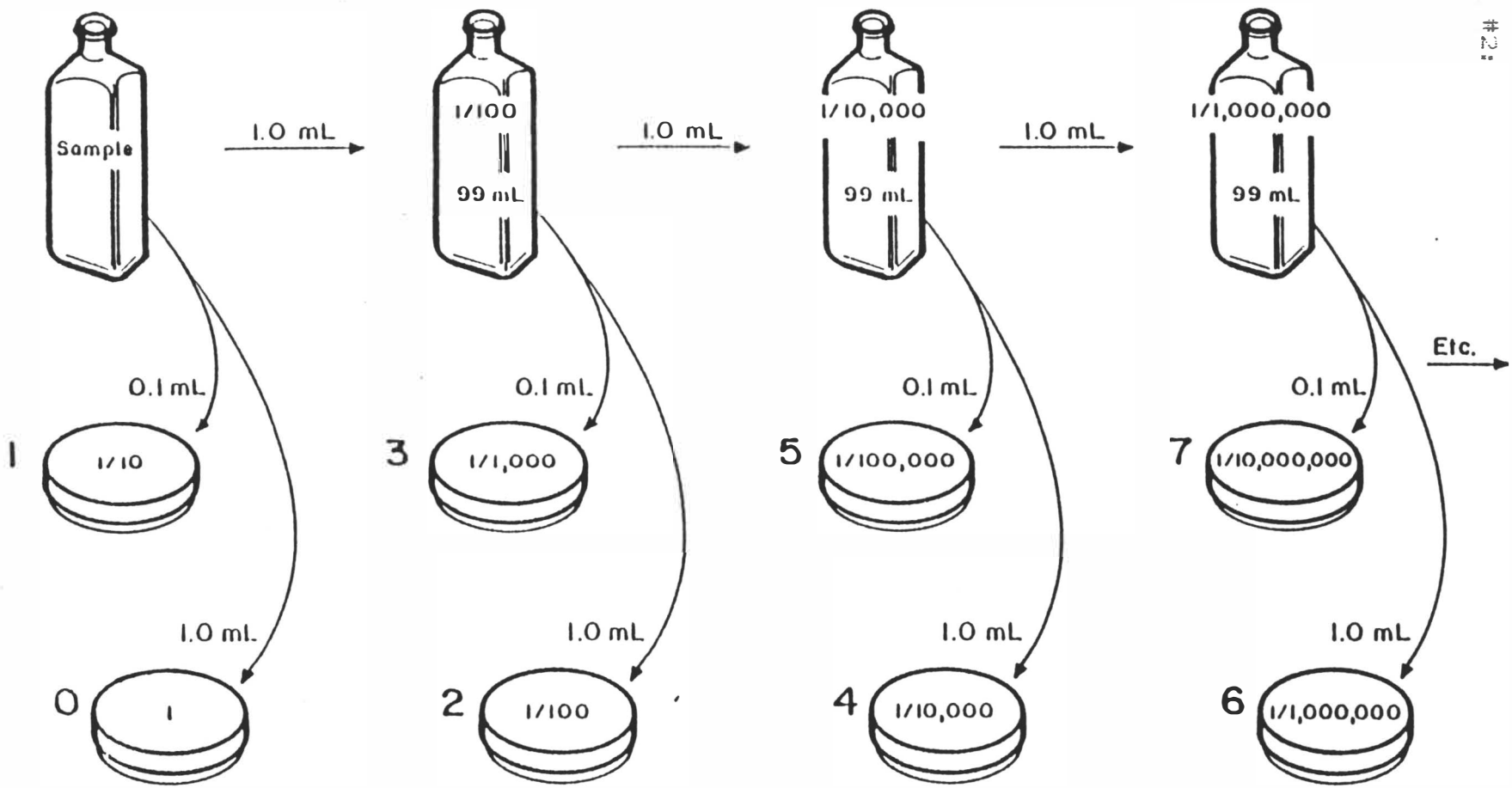


Figure 1

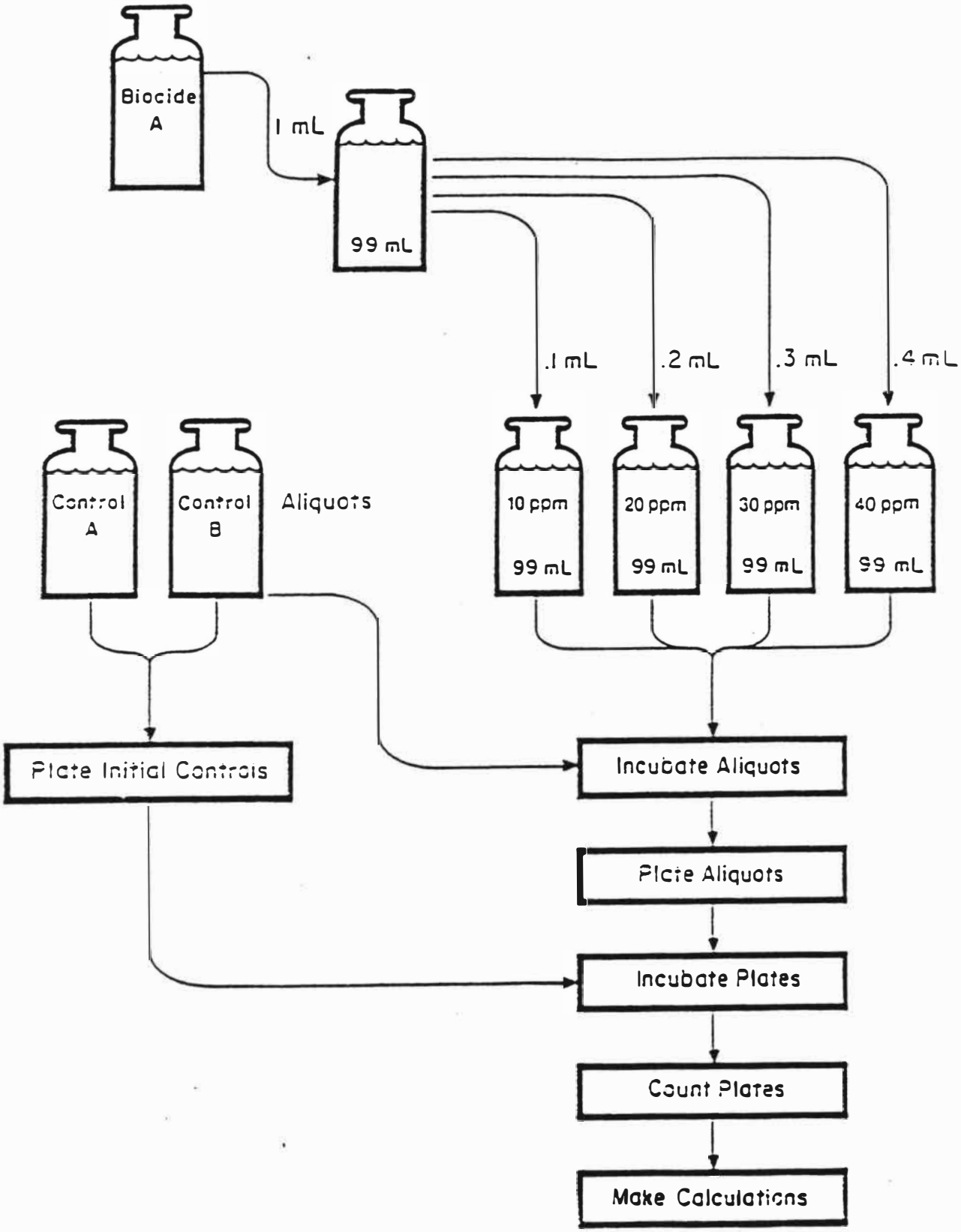
SONIFIER Cell Disruptor

1. Power Supply
2. Converter
3. Disruptor Horn
4. Timing Control
5. Output Control



Procedure Used When Making Dilutions for Quantitative Bacteriological Determinations

Appendix #5: Diagram of a Dilution Type Toxicant Evaluation



Appendix #4: Data for Biocide Treatment Runs.

Biocide Type	Treatment Dosage (ppm)	Plate count Run A	Run B	Avg.
MBT	10	159	147	153
	50	142	102	122
	100	74	102	88
Isothiazolin	10	198	181	190
	50	130	198	164
Control		465	482	473
Isothiazolin	100	91	113	102
Thiadiazine	10	181	128	153
	50	96	113	105
	100	57	74	65
Control		465	459	462
MBT	150	50	62	56
Isothiazolin	150	63	67	65
Thiadiazine	150	30	42	36
Control		503	550	527

* All counts were performed on the 10E-5 dilution plate.
* All counts are X 10E5

Appendix #5: Data for Ultrasonic Treatment Runs.

Power Level	Treatment Time (min)	Plate count Run A	Run B	Avg.
3	1	266	323	294
	3	259	238	249
	5	232	232	232
5	1	266	301	284
	3	249	170	208
	5	204	193	198
7	1	279	252	266
	3	142	232	187
	5	187	142	164
Control		338	354	346
3	7	228	234	231
	9	180	194	187
5	7	169	177	173
	9	105	91	98
7	7	116	108	112
	9	47	49	48
Control		408	390	399

* All counts were performed on the 10E-5 dilution plate.
* All counts are X 10E5