Use of electrical and magnetic fields in the control of bacterial biofilm  
by Susana M Fortun  

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in  
Electrical Engineering  
Montana State University  
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Abstract:  
Bacterial biofilm infections are less susceptible to antibiotic treatment than their planktonic counterparts. This has become a major health issue in many areas of a hospital and/or health care industry. The bioelectric effect was discovered by a team of Canadian scientists in 1994. The bioelectric effect is the treatment of the bacterial biofilm with antibiotic in the presence of an electrical current. This treatment has been found to produce a remarkable higher kill of the bacterial biofilm. This thesis replicated the original work done in Canada and from that base it goes into developing a dose response for the applied current in the presence and the absence of antibiotics. It shows that the effect is obtained with pure DC current and that the effect is not dependent on the direction of the current with respect to the nutrient flow. It also shows that the effect is only seen when the biofilm itself is treated with the DC current. We also have shown that an E-Field does not seem to have the same effect on the biofilm. Preliminary work was done using currents induced by AC fields and chemical manipulation of the nutrients. A mechanism for the bioelectric effect is proposed.
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of
Doctor of Philosophy
in
Electrical Engineering

MONTANA STATE UNIVERSITY-BOZEMAN
Bozeman, Montana

August 1997
APPROVAL

of a thesis submitted by

Susana M. Fortun

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduates Studies.

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(Date)

Approved for the Department of Electrical Engineering

Bruce R. McLeod, Ph.D.  
(Signature)  
(Date)

Approved for the College of Graduate Studies

Robert Brown, Ph.D.  
(Signature)  
(Date)
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Date: 8-5-77
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Bacterial biofilm infections are less susceptible to antibiotic treatment than their planktonic counterparts. This has become a major health issue in many areas of a hospital and/or health care industry. The bioelectric effect was discovered by a team of Canadian scientists in 1994. The bioelectric effect is the treatment of the bacterial biofilm with antibiotic in the presence of an electrical current. This treatment has been found to produce a remarkable higher kill of the bacterial biofilm. This thesis replicated the original work done in Canada and from that base it goes into developing a dose response for the applied current in the presence and the absence of antibiotics. It shows that the effect is obtained with pure DC current and that the effect is not dependent on the direction of the current with respect to the nutrient flow. It also shows that the effect is only seen when the biofilm itself is treated with the DC current. We also have shown that an E-Field does not seem to have the same effect on the biofilm. Preliminary work was done using currents induced by AC fields and chemical manipulation of the nutrients. A mechanism for the bioelectric effect is proposed.
INTRODUCTION

The second half of the twentieth century has seen many advances in the field of medicine. One of those advances is in bioengineered implants. During the last few decades, the bioengineers have given us prosthetic hip joints and internal fixation devices, vascular catheters, prosthetic heart valves, cardiac pacemakers, arterial grafts, in-dwelling urinary catheters, peritoneal dialysis catheters, contact lenses, penile prostheses, and intrauterine contraceptive devices among many others. These engineered innovations have prolonged our lives and/or restored a quality of life lost due to a disease. Unfortunately, this progress has been accompanied by the emergence of a new challenge to medicine -- implant associated infections that may not respond to conventional antibiotic treatment alone and that often require removal of the infected implant. [1,2]

Bacterial biofilm has been found to be the culprit in many of these implant associated infections that do not respond to conventional antibiotic treatment [3]. Medical equipment that is used in close contact to patients, such as dental drills, endoscope and contact lenses cases, are also prime environments for biofilm formation. Biofilms are bacterial populations enclosed in an exopolysaccharide matrix that adhere to each other and/or surfaces or interfaces [4].
Even though the study of infections caused by biofilms in the medical setting is still in its initial stage, the study of biofilms involved in corrosion [5], souring [6], and fouling [7,8] problems has a long history. The growth of biofilm in pipelines has been a major problem in industry not only because the biofilms slow down fluid flow but also because the biofilm is hard to clear due to its characteristic enhancement of resistance to biocides. There have been numerous hypotheses about the nature of this enhanced resistance. One of the early hypotheses was that the exopolysaccharide matrix was a charged matrix and that this matrix was responsible for binding antimicrobial agents before they have the opportunity to penetrate the biofilm [9]. Therefore, in the early 90’s, J.W. Costerton and his team at the University of Calgary, Calgary, Alberta, Canada, began a series of studies in order to determine if a low-strength electric field could affect the biocide in the biofilm and enhance biocide killing [10,11]. They found out that a low-strength dc-electric field in conjunction with a biocide would indeed reduce the biofilm population. These experiments were duplicated in England by Jana Jass and Hilary Lappin-Scott [12]. The British team, however, did not show as much killing of the biofilm.

It has been the intention of this study to duplicate the original work of J.W. Costerton and to extend the work in order to establish a dose response and to elucidate the mechanism of the enhancement of resistance to antibiotics observed in microbial biofilms.
THE BACTERIAL BIOFILM

Defining a biofilm

Biofilms are defined as matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces. This definition includes microbial aggregates and floccules and also adherent populations within the pore spaces of porous media [47]. Direct observations of living biofilms (by optical and other non-invasive physical methods) done by several research groups have revealed a complex structure for the biofilm. It is composed of an infrastructure made of a polysaccharide matrix that holds together an elaborate system of microcolonies. These microcolonies are connected by water channels that function as a primitive circulatory system bringing nutrients from the bulk fluid and removing metabolic products [13].

Figure 1: The bacterial biofilm.

It is understood today that biofilms are a distinct growth phase of bacteria which is quite different than the planktonic phase. Planktonic bacteria are those which are free
to move in their environment and have been the center of bacterial studies since Louis Pasteur's discoveries 15 decades ago. Biofilm forming bacteria are bacteria that adhere to surfaces. These bacteria are called sessile bacteria, and they are characterized by the production of a fibrous, highly hydrated, exopolysaccharide matrix whose chemical composition is species specific.

The biofilm is formed by the adhesion of planktonic bacteria to a surface. During the complex process of adhesion, the planktonic bacteria change their phenotype as a response to the surface [14]. During the early stages of biofilm formation, the sessile bacteria find themselves multiplying and forming colonies of single or multiple species. These cellular colonies and their exopolysaccharide will create the microniche of each bacterial biofilm. Different biofilm bacteria respond to their specific microniche in a different manner with different growth and adhesion patterns. Eventually, a structurally complex mature biofilm will develop.

Electron microscope studies and X-ray crystallographic analysis show the exopolysaccharide, also known as glycocalyx [15], to be an ordered array of fine fibers providing a thick, continuous, hydrated, polyanionic matrix around the cells [16]. This polysaccharide may contain one or more anionic uronic acids, and it is densely concentrated around the microcolonies of cells that have produced its polymer [17].
**Pseudomonas aeruginosa**

Because *P. aeruginosa* is the predominant autochthonous inhabitant of all naturally oligotrophic aquatic ecosystems, this remarkably adaptable bacterium is the most numerous organism on our planet [2]. *Pseudomonas aeruginosa* has emerged as a very important pathogen affecting a wide variety of patients whose defense against infection is compromised by trauma or by underlying disease [2].

*Pseudomonas aeruginosa* is a member of the genus *Pseudomonas*. *Pseudomonas* can be found in both soil and water. They are colonizers or pathogens of plants or animals, including man. Therefore, *Pseudomonas* are of medical importance. They are saprophytes with a low virulence which means that they are opportunists that produce a disease in patients with low or local resistance against infections. They may be found in infections at many body sites, the most common of which are the urinary tract, respiratory tract, wounds, and blood [18].

*Pseudomonas* are Gram-negative, asporogenous, rod-shaped bacterium, polar mono- or with polytrichous flagellation (18-20°). Their motility is at 18-20°. They are oxidase positive. They have oxidative glycolysis without gas formation, or no saccharolysis. They are catalase positive and indole negative. *P. aeruginosa* was first described by Schroeter in 1872 [19]. It usually forms a blue-green pigment, pyocyanin, which is often visible in growth media and is a diagnostic for this species. It most commonly exhibits rough, flat grayish colonies with uneven borders (R-form), but variants with a smooth, dome-shaped and whiter appearance (S-form) may be observed. The sheen is a diagnostic of *P. aeruginosa*. The species has a typical penetrating aromatic smell reminiscent of spoiled fish. The smell is due to o-amino-aceto-phenon
and is particularly pronounced on peptone-rich media. *P. aeruginosa* has but one flagellum.

Table 1. Common characteristics of Pseudomonas aeruginosa [19].

<table>
<thead>
<tr>
<th>COMMON CHARACTERISTICS OF PSEUDOMONAS AERUGINOSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram negative</td>
</tr>
<tr>
<td>Rod shaped (straight, asporogenous, .5 - 1.0 x 1.5 - 4.0 µm)</td>
</tr>
<tr>
<td>Strict aerobes (some species may utilize nitrogen or arginine as terminal acceptor anaerobically)</td>
</tr>
<tr>
<td>Motile by polar flagella</td>
</tr>
<tr>
<td>Oxidase positive</td>
</tr>
<tr>
<td>Oxidative metabolism (mostly saccharolytic, some nonsaccharolytic species, no gas formation from sugars)</td>
</tr>
<tr>
<td>Chemoorganotrophs (facultative chemolithotrophs rare)</td>
</tr>
<tr>
<td>Catalase positive</td>
</tr>
<tr>
<td>Growth with acetate as sole carbon source, most nonfastidious, few require growth factors</td>
</tr>
<tr>
<td>NO$_3$ reduced to NO$_2$ or molecular N$_2$</td>
</tr>
<tr>
<td>Many species accumulate poly-β–hydroxybutyrate (PBHB)</td>
</tr>
<tr>
<td>Some species produce pigments</td>
</tr>
<tr>
<td>Indole negative</td>
</tr>
<tr>
<td>58 - 70 mol% G + C</td>
</tr>
</tbody>
</table>
Pseudomonas aeruginosa Biofilm

Bacteria stick tenaciously to any surface in a liquid or aqueous environment [20]. They do so by means of a mass of tangled fibers of polysaccharides, or branching sugar molecules, that extend from the bacterial surface and form a feltlike “glycocalyx” surrounding an individual cell or a colony of cells [20]. The bacterial glycocalyx can be defined as those structures, of bacterial origin, that contain polysaccharides and are lying outside the integral elements of the outer membrane of Gram-negative cells and the peptidoglycan of Gram-positive cells [16]. Pseudomonas aeruginosa produces a thick flexible peripheral capsular glycocalyx [16]. The precise chemical nature of the glycocalyx varies with the sugar composition of the fibers [16] and, therefore, it differs from cell to cell and probably also at different stages in the life of the individual cell.

The exopolysaccharide secreted by Pseudomonas aeruginosa is composed of alginate [15]. Linker and Jones, in 1966, were the first ones to report the production of alginate in Pseudomonas aeruginosa [21]. Alginate is a linear copolymer of β-1,4-linked D-mannuronic acid and its C-5 epimer L-guluronic acid [14].
BIOFILM GROWTH PROCEDURES

Growth Chamber Design

The growth chamber is a cylindrical, straight sided glass beaker that was modified slightly in the glass blowing shop at Montana State University. The modification was the addition of a glass overflow nozzle that was set to be just above the level of the growth solution in which the biofilms were formed. A second, thin walled polycarbonate cylinder was made that would slip into the glass cylinder but would have a snug fit to the inner wall of the glass cylinder. Slots were cut into the polycarbonate cylinder to support the slides upon which the biofilms were grown. Eight such slides could be inserted in the growth chamber in this arrangement which gave sufficient biofilms to have four controls and four "experimentals" with each experimental run.

The growth chamber was connected to two peristaltic pumps with peristaltic pump heads. The growth chamber was magnetically stirred and was connected to the nutrients, buffer and dilution water by MasterFlex tubing. Dilution water was stored in a plastic garbage can which was continuously suffused with air by using a fish aquarium oxygen pump.
### Equipment

The equipment used for growing the biofilm is specified in the following table.

Table 2: Equipment List.

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Manufacturer</th>
<th>Model #</th>
<th>Qt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Chamber</td>
<td>MSU</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Peristaltic Pump</td>
<td>Cole - Parmer Instrument Co.</td>
<td>MasterFlex Model 7553-70; 6-600 RPM</td>
<td>1</td>
</tr>
<tr>
<td>Peristaltic Pump</td>
<td>Cole - Parmer Instrument Co.</td>
<td>MasterFlex Model 7553-80; 1-100 RPM</td>
<td>1</td>
</tr>
<tr>
<td>Pump Head</td>
<td>Cole - Parmer Instrument Co.</td>
<td>MasterFlex Model 7016-20</td>
<td>1</td>
</tr>
<tr>
<td>Pump Head</td>
<td>Cole - Parmer Instrument Co.</td>
<td>MasterFlex Model 7014-20</td>
<td>2</td>
</tr>
<tr>
<td>Stirrer</td>
<td>Fisher Scientific</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Tubing</td>
<td></td>
<td>MasterFlex</td>
<td></td>
</tr>
<tr>
<td>Garbage Can</td>
<td>Rubbermaid</td>
<td>34 gal.</td>
<td>1</td>
</tr>
<tr>
<td>Air Pump</td>
<td>Rolf C. Hagen Corp. Mansfield, MA 02048</td>
<td>Model Elite 802</td>
<td>1</td>
</tr>
</tbody>
</table>
Materials and Methods

The material and methods used for growing the biofilm in all the experiments presented in this work are specified in the following two tables.

Growth Media

Table 3: Nutrients and Chemicals List.

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>SOURCE</th>
<th>COMPONENT</th>
<th>AMOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>Fisher Biotech, Fisher Chemical, Fair Lawn, NJ 07410</td>
<td>NaH₂PO₄</td>
<td>13.63 g/l</td>
</tr>
<tr>
<td>Buffer</td>
<td>Fisher Biotech, Fisher Chemical, Fair Lawn, NJ 07410</td>
<td>KH₂PO₄</td>
<td>6.56 g/l</td>
</tr>
<tr>
<td>Substrate</td>
<td>Fisher Biotech, Fisher Chemical, Fair Lawn, NJ 07410</td>
<td>KNO₃</td>
<td>0.43 g/l</td>
</tr>
<tr>
<td>Substrate</td>
<td>Aldrich Chemical Co., Inc. Milwaukee, WI 53233</td>
<td>MgSO₄*7H₂O</td>
<td>0.032 g/l</td>
</tr>
<tr>
<td>Substrate</td>
<td>J.T. Baker Chemical Co. St. Louis, MO 63178</td>
<td>CaCO₃</td>
<td>0.032 g/l</td>
</tr>
<tr>
<td>Substrate</td>
<td>Sigma Chemical Co. St. Louis, MO 63178</td>
<td>Glucose</td>
<td>0.64 g/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>Sigma Chemical Co. St. Louis, MO 63178</td>
<td>(HOCOCH₂)₃N</td>
<td>1.28 g/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>J.T. Baker Chemical Co. St. Louis, MO 63178</td>
<td>(NH₄)₆Mo₇O₂₄*4H₂O</td>
<td>8.96 mg/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>J.T. Baker Chemical Co. St. Louis, MO 63178</td>
<td>ZnSO₄*7H₂O</td>
<td>0.91 g/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>Sigma Chemical Co. St. Louis, MO 63178</td>
<td>MnSO₄*H₂O</td>
<td>72.96 mg/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>J.T. Baker Chemical Co. St. Louis, MO 63178</td>
<td>CuSO₄*5H₂O</td>
<td>17.92 mg/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>J.T. Baker Chemical Co. St. Louis, MO 63178</td>
<td>Na₂B₄O₇*10H₂O</td>
<td>8.96 mg/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>J.T. Baker Chemical Co. St. Louis, MO 63178</td>
<td>Co(NO₃)₂*6H₂O</td>
<td>10.7 mg/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>Fisher Biotech, Fisher Chemical, Fair Lawn, NJ 07410</td>
<td>FeSO₄*7H₂O</td>
<td>1.018 g/l</td>
</tr>
</tbody>
</table>
Methods

A strain of *Pseudomonas aeruginosa* (maintained in the Montana State University-Bozeman (MSU) Center for Biofilm Research as ERC-1) was used for these experiments. The bacteria was allowed to colonize polycarbonate slides within a growth chamber (described above) for 3 days, forming a biofilm. The procedure is described below:

Table 4: Procedure

<table>
<thead>
<tr>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1: A sterile growth chamber, filled with buffered distilled water, 20 mg/l of glucose and various trace minerals (listed under materials) is inoculated with a 1 ml of frozen <em>Pseudomonas aeruginosa</em> culture. This &quot;batch phase&quot; continues for 24 hours with constant stirring.</td>
</tr>
<tr>
<td>Day 2: 24 hours after inoculation, buffer, substrate and dilution water are fed continuously into the reactor at a dilution rate of 30:1.</td>
</tr>
<tr>
<td>Day 3: Make sure the dilution water is flowing.</td>
</tr>
<tr>
<td>Day 4: Make sure the dilution water is flowing.</td>
</tr>
<tr>
<td>Day 5: After approximately 72 hours' growth, the polycarbonate slides, with the attached bacterial biofilm, are transferred aseptically to the reaction chambers for the DC electrical current experiments.</td>
</tr>
</tbody>
</table>
Figure 2: The growth chamber station.
Tobramycin is an antibiotic of the aminoglycosides family. Its origin is from *Streptomyces tenebrarius* ATCC 17920 and it was first reported produced in 1967. The chemical formula is O-3-Amino-3-deoxy-a-D-glucopyranosyl-(I; C - 46.24%, H - 7.98%, N - 14.98%, O - 30.80%. It is a single factor antibiotic that consists of about 10% nebramycin, the aminoglycosidic antibiotic complex produced by *Streptomyces tenebrarius* [22].

Figure 3: Tobramycin [22].
DC ELECTRIC FIELD AND BACTERIAL BIOFILM

Reaction Chamber Design

The reaction chamber went through several designs until all experimental parameters were met.

1. **Design I:** The reaction chambers were built from FisherBrand five-slide, fifty-gauge polyprolylene slide transporter boxes (3 x 1 in. [ca. 8 x 3 cm]; Fisher Scientific). To create a pathway for the nutrient flow through the chamber in a left-to-right horizontal direction, holes were drilled on either end of the box with a 1/8-in. (ca. 0.3175 cm) drill. The hole on the left end of the box (influent) was drilled near the bottom of the chamber in the center, and the hole on the right end of the box (effluent) was drilled near the top of the chamber in the center. The larger ends of two connectors (1/16 x 3/32 in. [ca. 0.16 x 0.239 cm]) were cut at the groove, inserted into the holes previously drilled, and fastened in place with silicone adhesive. Number 22-gauge, platinum wire electrodes were placed at either end of the chamber (inside the chamber). Notches were cut in the end of the chamber, allowing the electrodes to project from the chamber. A small strip of scotch tape was placed on the top edge of the chamber to help stabilize the electrodes.

2. **Design II:** The reaction chambers were the same ones from design I, but the electrodes were made from stainless steel 22-gauge wire.
3. **Design III**: The reaction chambers were rectangular boxes consisting of polycarbonate top (10.3 x 2.8 x 0.5 cm), bottom (10.3 x 2.8 x 0.5 cm), front (10.3 x 3.4 x 0.5 cm) and back (10.3 x 3.4 x 0.5 cm) slabs, and the sides slabs made of stainless steel. The stainless steel sides had three machined grooves for supporting the slides in place. The bottom, front, back and side slabs were joined together by stainless steel screws.

The top slab served as a cover and was held in place by two stainless steel, one sided thread pins placed at opposite ends on each side of the plate. The stainless steel sides served as plate electrodes. Two plug-in connectors were attached, one to each plate, in order for the plate electrodes to be connected to the electrical circuit. Each stainless steel side plate had two fluid flow connectors attached to it. The upper one (0.5 cm outside diameter) for effluent flow and the lower one (0.2 cm outside diameter) for the affluent flow. Side plates were interchangeable.

4. **Design IV**: The reaction chambers were similar to Design III, but the side plates were made of polycarbonate and the electrodes were made from stainless steel 22-gauge wire. These reaction chambers were made of polycarbonate slabs measuring 10.3 x 3.4 x 0.5 cm. The stainless steel 22-gauge wire projected from the side of the box in order that they could be attached to the electronic circuit by alligator clips. The lid was also modified in order to secure it to the box by four screws, one on each corner of the box. The side plates had only one fluid flow connector, either the affluent small port (0.2 cm outside diameter) or the effluent larger one (0.5 cm outside diameter).
5. **Design V:** The reaction chambers were machined from a solid, rectangular block of polycarbonate with outside dimensions of 20 x 40 x 84 millimeters. An end mill was used to remove the material in a volume measuring 16 x 34 x 70.5 millimeters (approximately 38 cm$^3$) as shown in figure 4. The fluid flow connectors are also shown in the figure. A 34 x 88 millimeter, rectangular piece of polycarbonate, 5.7 millimeters thick, was used to form the lid for each reactor chamber. The lid was made this thick in order to give solid support to the 22-gauge stainless steel wires that formed the electrical contacts to the liquid in the chamber. Two holes were drilled through the lid, one at each end, such that the 22-gauge wire would easily push through the holes and extend down into the exposure chamber. The diameter of the holes was chosen so the wires would fit snugly in the holes. To further prevent leakage around the electrodes, a thin sheet of sterilised soft rubber was placed under the entire bottom side of the lid (e.g. a rubber sealing gasket) and the electrodes were pushed through the rubber at the start of each experiment. The holes for the electrodes were positioned so that the electrodes extended down the distal end of the grooves that held the biofilm substratum centered and upright in the reaction chamber. The wire electrodes were cut to a length that exposed 32 millimeters of electrode in the chamber (e.g. extending from the lid to near the bottom of the chamber), plus approximately three centimeters of wire extending outside the box for the purpose of connecting the electrode to the power supply circuit. A sterile rubber gasket was fitted to the entire under-surface of the reaction chamber lid, and the lid was then fastened
to the chamber with six screws equally spaced along the long sides of the chamber (three on each side).

6. **Design VI**: The reaction chambers were the same as Design V, but two of them were connected in series.

The following table will summarize the design reaction chambers, and electrode’s material.

**Table 5: Different stages of the reaction chamber design.**

<table>
<thead>
<tr>
<th>Design #</th>
<th>Box Type</th>
<th>Electrode Type</th>
<th>Electrode Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Single</td>
<td>Wire</td>
<td>Platinum</td>
</tr>
<tr>
<td>II</td>
<td>Single</td>
<td>Wire</td>
<td>Stainless Steel</td>
</tr>
<tr>
<td>III</td>
<td>Single</td>
<td>Plates</td>
<td>Stainless Steel</td>
</tr>
<tr>
<td>IV</td>
<td>Single</td>
<td>Wire</td>
<td>Stainless Steel</td>
</tr>
<tr>
<td>V</td>
<td>Single</td>
<td>Wire</td>
<td>Stainless Steel</td>
</tr>
<tr>
<td>VI</td>
<td>Double</td>
<td>Wire</td>
<td>Stainless Steel</td>
</tr>
</tbody>
</table>

The following table will summarize the physical parameters of the different reaction chambers.

**Table 6: Physical dimensions of the different reaction chamber models.**

<table>
<thead>
<tr>
<th>Design #</th>
<th>Lenght (mm)</th>
<th>Width (mm)</th>
<th>Depth (mm)</th>
<th>Volume (ml³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>External</td>
<td>Internal</td>
<td>External</td>
<td>Internal</td>
</tr>
<tr>
<td>I</td>
<td>81</td>
<td>78</td>
<td>18</td>
<td>14.8</td>
</tr>
<tr>
<td>III</td>
<td>103</td>
<td>77</td>
<td>27.5</td>
<td>15</td>
</tr>
<tr>
<td>IV</td>
<td>103</td>
<td>77</td>
<td>27</td>
<td>16</td>
</tr>
<tr>
<td>V</td>
<td>83</td>
<td>71</td>
<td>29</td>
<td>16</td>
</tr>
</tbody>
</table>
Figure 4: Reaction chamber.
The equipment used in all the dc-current experiments described in this work is specified in the following table.

Table 7: Equipment used in the experiments.

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Manufacturer</th>
<th>Model #</th>
<th>Qt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammeter</td>
<td>John Fluke Manufacturing Co., Inc. Everett, WA</td>
<td>Digital Multimeter Model 8000A</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Keithley Instruments, Inc. Cleveland, Ohio 44139</td>
<td>Digital Multimeter Model 169</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Nilsson Electronic Labs, Inc. New York, NY</td>
<td>Analog Ammeter Model Weston 901</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>John Fluke Manufacturing Co., Inc. Everett, WA</td>
<td>Digital Multimeter Model 8012B</td>
<td>1</td>
</tr>
<tr>
<td>Power Source</td>
<td>Hewlett HP Packard</td>
<td>Model 6216A</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Hewlett HP Packard</td>
<td>Model 6217A</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sorensen</td>
<td>Q Norbatron Model QRB30-1</td>
<td>1</td>
</tr>
<tr>
<td>Peristaltic Pump</td>
<td>Cole - Parmer Instrument Co.</td>
<td>MasterFlex Model # 7553-80 1-100 RPM</td>
<td>2</td>
</tr>
<tr>
<td>Pump Head</td>
<td>Cole - Parmer Instrument Co.</td>
<td>MasterFlex Model 7013-20</td>
<td>7</td>
</tr>
<tr>
<td>Tubing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controller</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Vortex</td>
<td>Scientific Industries Inc., Bohemia, NY 11716</td>
<td>Genie 2 Model G-560</td>
<td>1</td>
</tr>
<tr>
<td>Pipette</td>
<td>Rainin</td>
<td>edp 2</td>
<td>1</td>
</tr>
<tr>
<td>Pipette</td>
<td>Gilson</td>
<td>Pipetman Model P 1000</td>
<td>1</td>
</tr>
</tbody>
</table>
**Materials and Methods**

The materials and methods used in all the dc-current experiments described in this work are specified in the following two tables.

**Material**

**Table 8: Nutrients and Chemical list.**

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>SOURCE</th>
<th>COMPONENT</th>
<th>AMOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>Fisher Biotech, Fisher Chemical, Fair Lawn, NJ 07410</td>
<td>NaH₂PO₄</td>
<td>13.63 g/l</td>
</tr>
<tr>
<td>Buffer</td>
<td>Fisher Biotech, Fisher Chemical, Fair Lawn, NJ 07410</td>
<td>KH₂PO₄</td>
<td>6.56 g/l</td>
</tr>
<tr>
<td>Substrate</td>
<td>Fisher Biotech, Fisher Chemical, Fair Lawn, NJ 07410</td>
<td>KNO₃</td>
<td>0.43 g/l</td>
</tr>
<tr>
<td>Substrate</td>
<td>Aldrich Chemical Co., Inc. Milwaukee, WI 53233</td>
<td>MgSO₄*7H₂O</td>
<td>0.032 g/l</td>
</tr>
<tr>
<td>Substrate</td>
<td>J.T. Baker Chemical Co. St. Louis, MO 63178</td>
<td>CaCO₃</td>
<td>0.032 g/l</td>
</tr>
<tr>
<td>Substrate</td>
<td>Sigma Chemical Co. St. Louis, MO 63178</td>
<td>Glucose</td>
<td>0.64 g/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>Sigma Chemical Co. St. Louis, MO 63178</td>
<td>(HOOCCH₂)₃N</td>
<td>1.28 g/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>J.T. Baker Chemical Co. St. Louis, MO 63178</td>
<td>(NH₄)₆Mo₇O₂₄*4H₂O</td>
<td>8.96 mg/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>J.T. Baker Chemical Co. St. Louis, MO 63178</td>
<td>ZnSO₄*7H₂O</td>
<td>0.91 g/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>Sigma Chemical Co. St. Louis, MO 63178</td>
<td>MnSO₄*H₂O</td>
<td>72.96 mg/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>J.T. Baker Chemical Co. St. Louis, MO 63178</td>
<td>CuSO₄*5H₂O</td>
<td>17.92 mg/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>J.T. Baker Chemical Co. St. Louis, MO 63178</td>
<td>Na₂B₄O₇*10H₂O</td>
<td>8.96 mg/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>J.T. Baker Chemical Co. St. Louis, MO 63178</td>
<td>Co(NO₃)₂*6H₂O</td>
<td>10.7 mg/l</td>
</tr>
<tr>
<td>Trace Elements</td>
<td>Fisher Biotech, Fisher Chemical, Fair Lawn, NJ 07410</td>
<td>FeSO₄*7H₂O</td>
<td>1.018 g/l</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>APOTHECON Bristol-Myers Squibb Co., Princeton, NJ 0854</td>
<td>Tobramycin</td>
<td>5 mg/l</td>
</tr>
</tbody>
</table>
### Methods

Table 9: Procedure for the experimental part.

<table>
<thead>
<tr>
<th>Day</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 4</td>
<td>Nutrients are prepared in 1000 ml flasks and autoclaved. Tubing and connectors are autoclaved. They are left overnight to cool.</td>
</tr>
<tr>
<td>Day 5</td>
<td>Slides are placed in their respective reaction chamber. There are 8 slides. The biofilm on one slide is scraped from the polycarbonate slide into a sterile beaker and 9 ml of sterile buffer solution is added. An additional 1 ml is added to the beaker after the first 9 ml with the biofilm in it has been poured back into the test tube. This extra 1 ml will rinse any biofilm left in the beaker and it will be added to the test tube. 8, 10x dilutions are made and plated into petri-dishes using a Ranin pipette. Plates are incubated at 35°C for 17 to 18 hours and counted. This count is referred to as the &quot;original count&quot;. Tobramycin in concentration of 5 mg/l is added to the original biofilm dilution and placed in a stirring water bath for 24 hours. Before the transfer of the slides, the salts, glucose and the antibiotic (when it is called for in the experiment), has been added to the nutrient flask in a sterile procedure using sterile syringes, needles and syringe filters. The tubing is connected to the flasks and the reactions chambers through peristaltic pumps to aid in the nutrients flow. The nutrients are allowed to flow in order to fill the reaction chambers. Once the reaction chambers are full, the transfer of the slides can occur. The slides are transferred using sterile techniques. Currents are set at the desired setting and the slides are left in the chamber for 24 hours.</td>
</tr>
<tr>
<td>Day 6</td>
<td>After 24 hours in the experimental chambers, the slides are retrieved one at a time. The biofilm from each slide is scraped using sterile procedures into a sterile beaker using tweezers and a stainless steel spatula. Serial dilutions are performed on the suspended cells up to 1 x 10^-8 dilutions and plated using the Ranin pipette. The plantonic culture with antibiotic is also diluted and plated. The petri-dishes are placed in an incubators at 35°C for 17 to 18 hours. The original count is counted and recorded.</td>
</tr>
<tr>
<td>Day 7</td>
<td>Plates are read and recorded.</td>
</tr>
</tbody>
</table>
The experiments were conducted in a series of six groups. All the procedures, nutrients, buffer and antibiotic quantities were kept constant. The only variation in each set is the one noted for the group. These groups are:

**Dose response:** In this set of experiments, the biofilm was subjected to different values of DC current in order to demonstrate a current dose response. We conducted this set of experiments in the presence of antibiotics and in its absence.

Table 10: Dose response experiments using antibiotics and box VI.

<table>
<thead>
<tr>
<th>Current (mA)</th>
<th>Experiment #</th>
<th>Qt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30, 32, 34, 35, 36, 37</td>
<td>6</td>
</tr>
<tr>
<td>.25</td>
<td>34, 47, 49</td>
<td>3</td>
</tr>
<tr>
<td>.5</td>
<td>30, 34, 35</td>
<td>3</td>
</tr>
<tr>
<td>.75</td>
<td>34, 47, 49</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>32, 34, 36, 39</td>
<td>4</td>
</tr>
<tr>
<td>1.5</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>32, 37, 38</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>32, 37, 38</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 11: Dose response experiments without antibiotics and box VI.

<table>
<thead>
<tr>
<th>Current (mA)</th>
<th>Experiment #</th>
<th>Qt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30, 32, 34, 35, 36</td>
<td>5</td>
</tr>
<tr>
<td>.5</td>
<td>30, 35</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>30, 35, 36</td>
<td>3</td>
</tr>
<tr>
<td>1.5</td>
<td>30, 35, 36</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>32, 34, 36</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>32, 35, 36, 37</td>
<td>4</td>
</tr>
</tbody>
</table>
Polarity: Polarity of the current with respect to the nutrient flow. In this set of experiments, all the parameters were kept constant including the antibiotic. The only parameter that changed was the positioning of the positive and negative electrode with respect to the nutrient flow.

Table 12: Polarity of the current with respect to the nutrient flow.

<table>
<thead>
<tr>
<th>Polarity</th>
<th>Experiment #</th>
<th>Qt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up Stream</td>
<td>39, 37, 38, 19, 25-29, 17, 24</td>
<td>12</td>
</tr>
<tr>
<td>Down Stream</td>
<td>39, 37, 38, 19, 25-29, 18</td>
<td>11</td>
</tr>
</tbody>
</table>

Slide substratum orientation: In this set of experiments, all parameters were kept constant with the exception of the slide orientation and the type of electrodes used.

Table 13: Slide orientation.

<table>
<thead>
<tr>
<th>Slide Location</th>
<th>Electrode Type</th>
<th>Experiment #</th>
<th>Qt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Straight</td>
<td>Wire</td>
<td>15, 17, 24, 25, 26, 29</td>
<td>6</td>
</tr>
<tr>
<td>Straight</td>
<td>Plate</td>
<td>11, 13, 12</td>
<td>3</td>
</tr>
<tr>
<td>Diagonal</td>
<td>Wire</td>
<td>15, 17</td>
<td>2</td>
</tr>
<tr>
<td>Diagonal</td>
<td>Plate</td>
<td>20, 21, 22</td>
<td>3</td>
</tr>
</tbody>
</table>

Serial chamber configuration: In this set of experiments, we used two reaction chambers connected in series. The electrodes were placed in the first reaction chamber, and the slide with the biofilm was placed in the second one. The experiments were conducted both in the up stream and down stream modes. All other parameters were kept constant.

Table 14: Serial chamber.

<table>
<thead>
<tr>
<th>Polarity</th>
<th>Experiment #</th>
<th>Qt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up Stream</td>
<td>25, 26, 27, 28, 29</td>
<td>5</td>
</tr>
<tr>
<td>Down Stream</td>
<td>25, 26, 27, 28, 29</td>
<td>5</td>
</tr>
</tbody>
</table>
E-Field exposure: The electrodes were placed outside the reaction chamber. They were held to the reaction chamber by scotch tape. The experiments were conducted both in the up stream and down stream modes. All other parameters were kept constant.

Table 15: E-Field/Polarity, 5 V.

<table>
<thead>
<tr>
<th>Polarity</th>
<th>Experiment #</th>
<th>Qt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up Stream</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Down Stream</td>
<td>19</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 16: E-Field/Dose response.

<table>
<thead>
<tr>
<th>Volts</th>
<th>Experiment #</th>
<th>Qt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.4</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>10.8</td>
<td>19</td>
<td>1</td>
</tr>
</tbody>
</table>

Buffer concentration: The concentration of buffer salts were changed in order to obtain a dose response with and without antibiotics.

Table 17: Buffer concentration with antibiotics and 2 mA.

<table>
<thead>
<tr>
<th>Buffer Concentration</th>
<th>Experiment #</th>
<th>Qt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>.5 B</td>
<td>47</td>
<td>1</td>
</tr>
<tr>
<td>RB</td>
<td>32, 37, 38</td>
<td>3</td>
</tr>
<tr>
<td>1.1 B</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>2 B</td>
<td>44, 50</td>
<td>2</td>
</tr>
<tr>
<td>3 B</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>4 B</td>
<td>48, 48, 50</td>
<td>3</td>
</tr>
<tr>
<td>5 B</td>
<td>41, 49, 49</td>
<td>3</td>
</tr>
</tbody>
</table>
Table 18: Buffer concentration with antibiotics and 0 mA.

<table>
<thead>
<tr>
<th>Buffer Concentration</th>
<th>Experiment #</th>
<th>Qt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>.5 B</td>
<td>47</td>
<td>1</td>
</tr>
<tr>
<td>RB</td>
<td>30, 32, 34, 35, 36, 37</td>
<td>6</td>
</tr>
<tr>
<td>1.1 B</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>2 B</td>
<td>44</td>
<td>1</td>
</tr>
<tr>
<td>3 B</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>4 B</td>
<td>47</td>
<td>1</td>
</tr>
<tr>
<td>5 B</td>
<td>41</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 19: Buffer concentration without antibiotics and 0 mA.

<table>
<thead>
<tr>
<th>Buffer Concentration</th>
<th>Experiment #</th>
<th>Qt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td>30, 32, 34, 35, 36</td>
<td>5</td>
</tr>
<tr>
<td>1.1 B</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>2 B</td>
<td>44</td>
<td>1</td>
</tr>
</tbody>
</table>
DC Circuit Design

The circuit design consisted of a dc power supply, an ammeter in series with the reaction chamber, a current controller for a more accurate delivery of the proper current, the reaction chamber and a voltmeter in parallel to it. All dc circuits were as shown in the following figure.

Figure 5: DC Circuit design.
AC ELECTRIC FIELD AND BACTERIAL BIOFILM

Equipment

The equipment used in all the ac-current experiments described in this work are specified in the following table.

Table 20: AC experiments. Equipment list.

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Manufacturer</th>
<th>Model #</th>
<th>Qt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solenoid</td>
<td>MSU</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Multimeter</td>
<td>John Fluke Manufacturing Co., Inc. Everett, WA</td>
<td>Digital Multimeter Model 1000</td>
<td>1</td>
</tr>
<tr>
<td>Oscilloscope</td>
<td>Tektronix Corp. Portland, OR</td>
<td>Tektronix 2220 60 MHz, Digital Storage</td>
<td>1</td>
</tr>
<tr>
<td>Function Generator</td>
<td>Beckman Industrial Corp. 630 Puente St. Brea, CA 92621</td>
<td>Circuitmate FG-2</td>
<td>1</td>
</tr>
<tr>
<td>Universal Counter</td>
<td>Beckman Industrial Corp. 630 Puente St. Brea, CA 92621</td>
<td>Circuitmate UC-10</td>
<td>1</td>
</tr>
<tr>
<td>Digital Magnetometer</td>
<td>Schonsteadt Instrument Co. Reston, VA</td>
<td>Model DM 2220-S5</td>
<td>1</td>
</tr>
<tr>
<td>Peristaltic Pump</td>
<td>Cole - Parmer Instrument Co.</td>
<td>MasterFlex Model # 7553-80 1-100 RPM</td>
<td></td>
</tr>
<tr>
<td>Pump Head</td>
<td>Cole - Parmer Instrument Co.</td>
<td>MasterFlex Model 7013-20</td>
<td></td>
</tr>
<tr>
<td>Tubing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vortex</td>
<td>Scientific Industries Inc., Bohemia, NY 11716</td>
<td>Genie 2 Model G-560</td>
<td>1</td>
</tr>
<tr>
<td>Pipette</td>
<td>Rainin</td>
<td>edp 2</td>
<td>1</td>
</tr>
<tr>
<td>Pipette</td>
<td>Gilson</td>
<td>Pipetman Model P 1000</td>
<td>1</td>
</tr>
</tbody>
</table>
Materials and Methods

The materials used in all the ac-field experiments are the same as the materials used for the dc-field experiments described in table 8.

The method used for the ac experiments is similar to the one used in the dc experiments, and it is described in table 9, but in this case the current is induced using either ac or direct connected techniques.

Experiments Design

The experiments were conducted in a series of two groups. All the procedures, nutrients, buffer and antibiotic quantities were kept constant. The only variation in each set is the one noted for the group. These groups are:

1. **AC-Coupled:** A cylindrical solenoid was fabricated in the Montana State University material shop to our specifications. It was made of PVC pipe (110.2 mm outside diameter and 100.1 mm internal diameter) and twenty turns of varnish coated magnetic wire were placed in the middle section of the pipe. The wire was soldered to two connectors for easy connection to the system. The reaction chamber was placed in the center of the cylindrical solenoid. The chamber was kept in place by affixing it to a polycarbonate semicircle that was machined to fit in the center of the solenoid.

2. **Direct-Connected:** The reaction chamber was connected to the function generator by the wire electrodes.
Figure 6: AC-Coupled circuit design.
Figure 7: Direct connected circuit design
RESULTS

DC EXPERIMENTS

All experiments described in this thesis were conducted under standard conditions as stated under the following table unless it is noted otherwise.

Table 21: DC experiments. Standard conditions

<table>
<thead>
<tr>
<th>Electrode material</th>
<th>Stainless steel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrode type</td>
<td>Wire</td>
</tr>
<tr>
<td>Electrode location</td>
<td>Inside chamber</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Tobramycin</td>
</tr>
<tr>
<td>Antibiotic concentration</td>
<td>5 mg/l</td>
</tr>
<tr>
<td>Polarity</td>
<td>Up stream</td>
</tr>
<tr>
<td>Slide location</td>
<td>Straight</td>
</tr>
<tr>
<td>Nutrient flow</td>
<td>Up stream</td>
</tr>
</tbody>
</table>

Figure 8: Standard reaction chamber set up.
This set of experiments show that the material of the electrodes is not an issue in obtaining the increased killing of the bacterial biofilm. A series of experiments were conducted in order to compare the effect using platinum electrodes and the effect using stainless steel electrodes.

Table 22: Electrode material results.

<table>
<thead>
<tr>
<th></th>
<th>PLATINUM</th>
<th>STAINLESS STEEL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STRAIGHT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DIAGONAL</td>
<td></td>
</tr>
<tr>
<td>log (E/PC)</td>
<td>5.95</td>
<td>2.12</td>
</tr>
</tbody>
</table>

NOTE: E: Experiment and PC: Positive Control
Dose Response With Antibiotics

Dose response with antibiotic experiments were performed at several different stages of the research. They were done using different models of the reaction chamber, but all of them showed a very marked dose response to different current intensities. These experiments were conducted using 5 mg/l of Tobramycin, up stream polarity and our standard equipment set up unless otherwise indicated.

The series of tables and graphs below show that starting at 2 mA, the killing of the bacterial biofilm is complete. This reduction is of 6 logs of magnitude. This result has been so constant throughout all our experiments that it became one of our standards in the analyzing of the data.

Table 23: Dose response summary of results with box I and platinum-wire electrodes.

<table>
<thead>
<tr>
<th></th>
<th>.5 mA</th>
<th>1 mA</th>
<th>5 mA</th>
<th>7.5 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log (E/PC)</td>
<td>2.56</td>
<td>6.03</td>
<td>6.28</td>
<td>6.53</td>
</tr>
</tbody>
</table>

Table 24: Dose response summary of results with box IV and SS-wire electrodes.

<table>
<thead>
<tr>
<th></th>
<th>.5 mA</th>
<th>1 mA</th>
<th>1.5 mA</th>
<th>2 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log (E/PC)</td>
<td>1.28</td>
<td>3.61</td>
<td>3.56</td>
<td>5.58</td>
</tr>
</tbody>
</table>

Table 25: Dose response summary of results with box VII, SS-wire electrodes and current controller.

<table>
<thead>
<tr>
<th></th>
<th>0 mA</th>
<th>.25 mA</th>
<th>.5 mA</th>
<th>.75 mA</th>
<th>1 mA</th>
<th>1.5 mA</th>
<th>2 mA</th>
<th>3 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log (E/PC)</td>
<td>1.20</td>
<td>2.18</td>
<td>1.61</td>
<td>3.52</td>
<td>4.10</td>
<td>3.56</td>
<td>6.05</td>
<td>5.88</td>
</tr>
</tbody>
</table>
ANTIBIOTIC/FIELD - DOSE RESPONSE

![Graph showing dose response with antibiotic curve.](image)

Figure 9: Dose response with antibiotic curve.
**Dose Response Without Antibiotics**

These series of experiments show an interesting “bump” at around 2 mA. The killing of the bacterial biofilm stays low in the absence of antibiotics but at 2 mA there is a sudden increase in the killing. This increase suggests that at that current intensity there is a total electric effect in the biofilm that causes about a one log reduction of the resistance of the bacterial biofilm to be killed.

Table 26: Dose response summary of results with box II and SS-plate electrodes.

<table>
<thead>
<tr>
<th></th>
<th>1 mA</th>
<th>2 mA</th>
<th>4 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log (E/PC)</td>
<td>0.88</td>
<td>5.13</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Table 27: Dose response summary of results with box IV and SS-wire electrodes.

<table>
<thead>
<tr>
<th></th>
<th>1 mA</th>
<th>2 mA</th>
<th>4 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log (E/PC)</td>
<td>-0.03</td>
<td>0.33</td>
<td>2.67</td>
</tr>
</tbody>
</table>

Table 28: Dose response summary of results with box VII and SS-wire electrodes.

<table>
<thead>
<tr>
<th></th>
<th>0 mA</th>
<th>.5 mA</th>
<th>1 mA</th>
<th>1.5 mA</th>
<th>2 mA</th>
<th>3 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log (E/PC)</td>
<td>0.00</td>
<td>0.03</td>
<td>-0.18</td>
<td>1.11</td>
<td>1.72</td>
<td>1.08</td>
</tr>
</tbody>
</table>
Figure 10: Dose response without antibiotic curve.
Polarity

This set of experiments was designed to investigate the possibility of the production of antimicrobial chemicals at the positive electrode which might travel in the same direction as the flow of nutrients. As can be observed in the following tables, there is no statistical difference in the increased kill of the bacterial biofilm.

All these experiments were conducted using our standard 5 mg/l of Tobramycin and all other parameters were kept constant unless otherwise stated.

Table 29: Results of polarity experiments using box model IV, 1 mA, wire- S.S. electrodes. For detailed data see Appendix B.

<table>
<thead>
<tr>
<th>Mean log (E/PC)</th>
<th>UP STREAM</th>
<th>DOWN STREAM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.05</td>
<td>4.63</td>
</tr>
</tbody>
</table>

Table 30: Results of polarity experiments using box model VII, wire-S.S. electrodes.

<table>
<thead>
<tr>
<th></th>
<th>1 mA</th>
<th>2 mA</th>
<th>3 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP STREAM</td>
<td>DOWN STREAM</td>
<td>UP STREAM</td>
</tr>
<tr>
<td>Mean log (E/PC)</td>
<td>6.17</td>
<td>6.17</td>
<td>5.98</td>
</tr>
</tbody>
</table>
Figure 11: Polarity set up.
Slide Location

These experiments came to be serendipitous. After the tremendous success of our first box model, we designed a more sturdy model with stainless steel plates as electrodes. The idea was to have a more uniform field between the electrodes. The results, as shown in table 32, were quite different than the ones obtained with our previous box model and wire electrodes (see table 23).

A series of four experimental sets were conducted in order to investigate the effect. In the first set, wire electrode were used and the coupon was in the “straight” position as shown in the bottom figure in figure 12. In this case the experiment showed total kill. In the second set, wire electrode were also used but in this case the coupon was in the “diagonal” position as shown in the upper figure in figure 12. This case showed a reduce effect in the killing (see tables 33 and 34). The third set consisted of stainless steel plate electrodes and the coupon was placed in the “straight” position. These experiments showed that the bacterial kill was significantly smaller (see table 31) than the fourth set of experiments, in which the same plates were used but the coupon was in the “diagonal” position (see table 32).

Table 31: Summary using box II, SS-plates electrodes with the slide in the straight position.

<table>
<thead>
<tr>
<th>Mean log (E/PC)</th>
<th>1 mA</th>
<th>3 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.64</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Table 32: Summary using box II, SS-plates electrodes with the slide in the diagonal position.

<table>
<thead>
<tr>
<th></th>
<th>1 mA</th>
<th>2 mA</th>
<th>4 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log (E/PC)</td>
<td>0.88</td>
<td>5.13</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 33: Summary of results using box III, platinum-wire electrodes with the slide in the straight and diagonal position. Current, 1 mA.

<table>
<thead>
<tr>
<th></th>
<th>STRAIGHT</th>
<th>DIAGONAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log (E/PC)</td>
<td>5.95</td>
<td>2.12</td>
</tr>
</tbody>
</table>

Table 34: Summary of results using box IV, SS-wire electrodes with the slide in the straight and diagonal position. Current, 1 mA.

<table>
<thead>
<tr>
<th></th>
<th>STRAIGHT</th>
<th>DIAGONAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log (E/PC)</td>
<td>5.05</td>
<td>2.74</td>
</tr>
</tbody>
</table>

Figure 12: Slide location for the "Diagonal" setup (top) and for the "Straight" setup (bottom).
Serial Chambers

These experiments were designed in order to investigate the possibility of a pretreatment of the antibiotic. The hypothesis was that the electrical current may change the polarity of the antibiotic making it able to penetrate the polysaccharide matrix and kill the bacteria inside the biofilm.

The experimental set up was two reaction chambers in series with the wire electrodes placed in the standard position in the first box and the coupon with the bacterial biofilm in the second chamber (see figure 13). The nutrient solution, with the antibiotic, was allowed to flow in the standard direction through first chamber then into the second.

As shown in the table below, the experiments did not show a highly increased killing of the bacterial biofilm in either polarity.

Table 35: Summary of results using box VI, SS-wire electrodes with the slide in the straight position. Current, 2 mA

<table>
<thead>
<tr>
<th></th>
<th>UP STREAM</th>
<th>DOWN STREAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log (E/PC)</td>
<td>1.71</td>
<td>1.79</td>
</tr>
</tbody>
</table>
Figure 13: Serial reaction chamber set up.
These experiments were designed to investigate the possibility of the bacterial biofilm being killed by the electrical field alone. The results (see table 35 and 36) show that it is not probable that the E-field causes the enhanced killing in the presence of antibiotics.

The electrical field was obtained by placing the electrodes outside the box ends and held in place with scotch tape (see Figure 14).

Table 36: Summary using box IV, SS-wire electrodes, antibiotics and the slide in the straight position. Up stream polarity.

<table>
<thead>
<tr>
<th>Voltage</th>
<th>Mean log (E/PC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.4 V</td>
<td>1.71</td>
</tr>
<tr>
<td>10.8 V</td>
<td>1.79</td>
</tr>
</tbody>
</table>

Table 37: Summary using box V, SS-wire electrodes, antibiotics and the slide in the straight position. Voltage: 5 V.

<table>
<thead>
<tr>
<th>Voltage</th>
<th>UP STREAM</th>
<th>DOWN STREAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log (E/PC)</td>
<td>1.36</td>
<td>0.78</td>
</tr>
</tbody>
</table>
Figure 14: E-Field experiments set up.
Buffer Concentrations

In this series of experiments the salt concentration in the buffer was varied as noted for each experiment. All other parameters were kept constant.

Table 38: Buffer concentrations results: antibiotics and a current of 2 mA.

<table>
<thead>
<tr>
<th>.5 B</th>
<th>RB</th>
<th>1.1 B</th>
<th>2 B</th>
<th>3 B</th>
<th>4 B</th>
<th>5 B</th>
</tr>
</thead>
<tbody>
<tr>
<td>log (E/PC)</td>
<td>5.66</td>
<td>6.05</td>
<td>3.85</td>
<td>3.65</td>
<td>6.44</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Table 39: Buffer concentrations results: antibiotics and no current.

<table>
<thead>
<tr>
<th>.5 B</th>
<th>RB</th>
<th>1.1 B</th>
<th>2 B</th>
<th>4 B</th>
<th>5 B</th>
</tr>
</thead>
<tbody>
<tr>
<td>log (E/PC)</td>
<td>0.83</td>
<td>1.10</td>
<td>3.11</td>
<td>1.38</td>
<td>1.33</td>
</tr>
</tbody>
</table>

Table 40: Buffer concentrations results: no antibiotics and no current.

<table>
<thead>
<tr>
<th>RB</th>
<th>1.1 B</th>
<th>2 B</th>
</tr>
</thead>
<tbody>
<tr>
<td>log (E/PC)</td>
<td>0.00</td>
<td>-0.56</td>
</tr>
</tbody>
</table>

pH

It was found that in those experiments done with antibiotics, a current of 2 mA, and regular buffer concentration, the pH would go down to a value of 4 when the experiments were conducted with the positive electrode up stream. If they were conducted with the positive electrode down stream, then the pH would go up to a value of 11.
In order to investigate this effect, experiments were conducted with a nutrient solution whose pH was changed to 9 and to 4.25. The results are shown in the following tables.

Table 41: pH 9.

<table>
<thead>
<tr>
<th></th>
<th>Anti/No Field</th>
<th>Anti/Field - 2 mA</th>
<th>Anti/Field - .5 mA</th>
<th>No Anti/No Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>log (E/PC)</td>
<td>1.49</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 42: pH 4.25.

<table>
<thead>
<tr>
<th></th>
<th>Anti/No Field</th>
<th>No Anti/No Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>log (E/PC)</td>
<td>-0.51</td>
<td>1</td>
</tr>
</tbody>
</table>

A series of experiments were designed in order to investigate if the decrease or increase of the pH was due to the electrical current or it was due to the biological part of the experiments. The same experiments were conducted, but the biofilm was not introduced into the system. The results are shown in the following table.

Table 43: pH measurements with regular buffer and no biofilm.

<table>
<thead>
<tr>
<th>ORIGINAL</th>
<th>Anti/No Field</th>
<th>Anti/Field - 2 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.8</td>
<td>6.8</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 44: pH measurements with five times the regular buffer concentration (SB) and no biofilm.

<table>
<thead>
<tr>
<th>ORIGINAL</th>
<th>Anti/No Field</th>
<th>Anti/Field - 2 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1</td>
<td>7.1</td>
<td>6.4</td>
</tr>
</tbody>
</table>
AC EXPERIMENTS

AC-Coupled

These experiments were designed to investigate the possibility of inducing the current through the bacterial biofilm using magnetic fields. A solenoid was used in order to induce the current through the system. The reaction chamber was placed in the center on the coils and all other parameters were kept constant unless otherwise noted.

The table below shows that at 40 mV there was an increased in the killing of the bacterial biofilm.

Table 45: Summary using box I, solenoid field and a frequency of 24 MHz.

<table>
<thead>
<tr>
<th>Voltage (mV)</th>
<th>10</th>
<th>40</th>
<th>80</th>
<th>100</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log (E/PC)</td>
<td>0.63</td>
<td>1.87</td>
<td>1.13</td>
<td>-0.31</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Direct-Connected

Table 46: Summary using box I, direct connected and a frequency of 20 MHz.

<table>
<thead>
<tr>
<th>Current (mA)</th>
<th>1 mA</th>
<th>3 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean log (E/PC)</td>
<td>1.52</td>
<td>0.31</td>
</tr>
</tbody>
</table>
DISCUSSION

Literature Review

The study of the effect of a DC electrical current through a biofilm, in the presence of an antimicrobial agent, started with a study done by Dr. Costerton’s group in the University of Calgary, Calgary, Alberta, Canada.

Their first paper published in July of 1992 described their investigation using a DC electrical current in the presence of an antibiotic. They used a modified Robbins device (MRD) which was altered by incorporating a platinum wire electrode at the base of the device (to be used as one of the electrodes) while the other electrode was the stainless steel sample stud. The electric field used was 1.5 V/cm and a current density of 15 μA/cm². The polarity was changed every 64 seconds, so that the electrodes alternated as anode and cathode. They used four different types of organisms: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus epidermis*, and *Candida albicans*. The antimicrobial agents used were Tobramycin, Ciprofloxacin and Cycloheximide. They reported a total kill of *P. aeruginosa* in the presence of antibiotics (at 8 MIC) and a dc field after 12 hours of exposure [11].

Their second paper, published in November 1992, described their study using the modified Robbins device (MRD) and an electric field (12 V/cm) with a current density of 2.1 mA/cm². The polarity was altered every 64 seconds. In this series of experiments they used Kathon, Quaternary Ammonium Compounds and Glutaraldehyde as biocides. They
showed approximately a 4 log bacterial reduction using current and glutaraldehyde and a 5 log reduction in the presence of quaternary ammonium compounds [10].

Costerton's third paper, published in December 1994, described their work using a newly designed chamber. They worked with Tobramycin. The flow cell was composed of three stainless steel electrodes that allowed them to extend their study to ascertain if the effect was applicable to all electrode surfaces and to material placed between the electrodes. They changed polarity every 64 seconds. Costerton et. al. reported a 6 log increase in killing in these experiments [23].

The fourth paper in this area of study was done by Jana Jass at the Department of Biological Sciences, Hatherly Laboratories, University of Exeter, Exeter, UK. Dr. Jass used an electrical colonization cell designed by her group for these experiments. They designed this chamber for the precise purpose that the biofilm was formed on a surface away from the electrodes, in order to avoid electrochemical and mechanical disturbances, but with the biofilm staying in the path of the electrical current. They used two parallel 5.31 cm² circular stainless steel plate electrodes. Dr. Jass used several dc electrical current densities in her experiments, but all of them were done changing polarity every 32 seconds. They reported only 1 log of increased killing [12].

The following table summarizes their results. The summary includes only the data using *P. aeruginosa* and, with the exception of the second paper which used glutaraldehyde as an antimicrobial agent, the rest used Tobramycin as the antibiotic.
Table 47: Comparison between a summary from the four papers that preceded the work in our laboratories and the work done in our laboratories. CFU (Colonies Forming Units).

<table>
<thead>
<tr>
<th>Electrode Material</th>
<th>Biofilm/electrical current path</th>
<th>Current Density (I) (mA/cm²)</th>
<th>Frequency (cycles/sec)</th>
<th>Anti. Conc. (mg/l)</th>
<th>Time (hours)</th>
<th>Decrease in CFU (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Costerton, et.al.</td>
<td>S.S./Platinum Perpendicular</td>
<td>0.015</td>
<td>.0156</td>
<td>8</td>
<td>12</td>
<td>4.5</td>
</tr>
<tr>
<td>Costerton, et.al.</td>
<td>S.S./Platinum Perpendicular</td>
<td>2.1</td>
<td>.0156</td>
<td>5 ppm</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>Costerton, et.al.</td>
<td>S.S. Parallel</td>
<td>1.7</td>
<td>.0156</td>
<td>5</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>Jass, et.al.</td>
<td>S.S. Perpendicular</td>
<td>9</td>
<td>.03125</td>
<td>10</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>Fortun, et.al.</td>
<td>S.S. Parallel</td>
<td>.36</td>
<td>DC</td>
<td>5</td>
<td>24</td>
<td>6</td>
</tr>
</tbody>
</table>

**Previous Mechanisms of Antimicrobial Resistance in Biofilms**

There have been various suggestions as to the mechanisms responsible for the recalcitrance of bacterial biofilms towards chemical and antibiotic treatments. As we shall see, evidence exists to support the validity of each hypothesis. In view of the complexity of a biofilm consortium and the wide range of chemical entities, it is unlikely that any single mechanism underlies resistance. Rather, the processes may apply to differing extents according to the nature of the biofilm, its age and its locations as well as the chemical antimicrobial used.

Some hypotheses concentrate upon a role for the exopolysaccharide in moderating access of biocides to the sessile cells. Others suggest that the limited availability of key nutrients within the biofilm imposes a slowing of the specific growth rate and expression of phenotypes which are not typical of planktonic cells in the same medium. A third
group suggests that reaction-diffusion interactions will inhibit the biocide action upon the biofilm.

Central to all of these hypotheses is the principle that attached cells within a biofilm differ significantly from their planktonic laboratory grown counterparts.

Nichols et. al. report that in a physiological buffer solution, tobramycin binds to alginate and to exopolysaccharides isolated from two mucoid strains of *Pseudomonas aeruginosa* and that the binding to alginate quantitatively accounts for the diffusion reported previously by Slack et. al. [24].

Kumon et. al. examined the role of exopolysaccharides as a barrier against antimicrobial penetration [25]. Their experiments showed that the penetration of the positively charged hydrophilic drugs, like aminoglycosides and polypeptides, was markedly inhibited by the exopolysaccharides tested. But the penetration of β-lactams, quinolones, and macrolides was not inhibited.

Other studies, however, have demonstrated that there is no difference in antibiotic susceptibilities between slime producing and non-slime producing bacteria. Gristina et. al. examined the susceptibility of *S. epidermis* biofilms [26]. He did not observe any difference between the two strains, suggesting that the presence of slime was not of significance in antibiotic penetration. Similarly, Evans et.al. showed no difference in tobramycin control of intact and resuspended biofilms of *E. coli* [27]. But Evans et. al. also assessed the susceptibility towards the fluoroquinolone antimicrobial ciprofloxacin of mucoid and non-mucoid strains of *P. aeruginosa* biofilm. In this set of experiments,
possession of a mucoid phenotype was associated with decreases in susceptibility which
were no longer apparent when the biofilm cells were removed from the glycocalyx. These
data suggest that ciprofloxacin interacts in some form with the exopolysaccharide [28].

The physiology of biofilm cells is extremely complex and is quite different from
their planktonic forms [29]. Recent research has shown that the metabolic activity of the
cells in a biofilm is controlled by the phenotypic expression of specific genes that are
depressed by adhesion to the surface, by conditions at the surface, or by growth in the
biofilm mode [14].

Biofilm bacteria live in microbial communities where the availability of nutrients
and the concentrations of end products depend, in part, on the metabolic activity of
adjacent cells. The restriction of the availability of certain essential nutrients is known to
influence the physiology of bacterial pathogens which, in turn, can profoundly affect their
susceptibilities to antibiotics [15].

Anwar et. al. have suggested a mechanism by which, when the biofilm is exposed
to antibiotics, the planktonic and surface biofilm cells are quickly deactivated. These cells
are actively growing and are very susceptible to the antibiotic. The amount of antibiotic
entering these cells is probably greater than the amount needed to inactivate them. This
excess of antibiotics will probably be destroyed by antibiotic-degrading enzymes. This
results in a significant decrease in the amount of antibiotic available to kill the biofilm
cells that are embedded in the thick exopolysaccharide matrix. This exopolysaccharide is
negatively charged and is known to function as an ion-exchange resin capable of binding
large numbers of antibiotic molecules. This will reduce the rate and number of antibiotic molecules that will reach the embedded biofilm cells, but the embedded biofilm cells are not actively engaged in cell division and are smaller in size. Slow growing cells are generally less susceptible to antibiotics. Under these circumstances, embedded biofilm cells may have sufficient time to switch on the expression of antibiotic-resistant factors like antibiotic-degrading enzymes [31] and, therefore, avoid the toxic effects of the antibiotic.

Much work has been done postulating the mechanisms already discussed, but it was not until the last few years that mathematical models have been proposed to elucidate the resistance to antimicrobial agents by bacterial cells in a biofilm. [31, 32, 33, 34, 35, 36].

Stewart's study of these theoretical systems supported both mechanisms as possible explanations for the reduced susceptibilities of biofilms microorganisms to antimicrobial agents. If a reaction-diffusion mechanism is responsible for the decrease of antibiotic efficacy, then the efficacy of the antimicrobial agent will be influenced by the strength of its action as a disinfectant and by its reactivity with the microbial system.

There is one more paper that should be mentioned. Julia Vrany, in her Master of Science thesis, clearly showed that the biofilm provided very little transport limitation for the antibiotics. This suggests that reduced susceptibility may be due to other factors [37].
Proposed Theory of the Mechanism

I am proposing a two stage mechanism for the resistance of *Pseudomonas aeruginosa* biofilm to Tobramycin: The biofilm resistance in the exopolysaccharide matrix and the resistance at the bacteria’s outer membrane surface level.

It has been shown that tobramycin does bind to the exopolysaccharide matrix of the biofilm. However, binding and consequent inhibition of diffusion cannot account for antibiotic resistance within the microcolonies and biofilm [38]. The viscosity and gel-forming properties of alginate are determined largely by its affinity for divalent cations, particularly Ca$^{2+}$ [39] and Mg$^{2+}$, which control the degree of cross-linking. Experiments have shown that in the absence of these cations the exopolysaccharide looses its viscosity and structure [15]. Therefore, the first phase of the bioelectric effect may be to break or cause holes to appear in the exopolysaccharide matrix by pulling those cations away from it and, just as a building crumbles when the infrastructure is taken away or badly damaged, the "cities" or microcolonies inside the biofilm will crumble and the cells will be exposed to the antibiotic.

The second phase happens at the bacteria’s membrane surface. It has been well documented that there is an antagonism of aminoglycosides by divalent cations [40, 41]. Hancock proposed, in 1981, that the uptake and killing of the biofilm by aminoglycosides requires interaction with an Mg$^{2+}$ binding site at the outer membrane [42]. In later work Hancock and coworkers suggested a pathway for antibiotics to penetrate the cells in
which polycationic antibiotics interact with a site on the outer membrane at which Mg$^{2+}$ noncovalently cross-bridges adjacent lipopolysaccharides molecules [43, 44].

Raulston et. al. concluded that magnesium protects cells if initially present in the environment [45]. There is also evidence that Mg$^{2+}$ ions are involved in the K-Ca gating [46].

I am proposing that the exopolysaccharide matrix with plenty of Mg$^{2+}$ ions does not allow the antibiotic to penetrate the cells because the Mg$^{2+}$ is occupying the sites of penetration, but when the biofilm is treated with antibiotics and a DC current, the Mg$^{2+}$ will be released and carried away from the cell membrane and the antibiotic can bind to and penetrate the membrane, thus destroying the cells.

The experiments conducted in our laboratories, and described in this thesis, show that there is a dose response with respect to the current passing through the biofilm. Our results show that at approximately a current density of 0.36 mA/cm$^2$, in the presence of antibiotics, the biofilm is totally killed. In order to prove that the biofilm was actually killed and not just detached and carried away with the nutrient flow, the nutrients in the reaction chamber were plated and counted (see experiment # 39). They were compared to the standard plating of the biofilm on the coupon. The results were very similar, proving that the biofilm is actually dead and not just detached. It is interesting to note that at the same current density, without the presence of antibiotics, an increase in killing was noted. The material of the electrode did not make a difference in the results.
These results bring support to the theory discussed above by showing that a threshold must be reached before the Mg$^{2+}$ or the Ca$^{2+}$ can be taken from the exopolysaccharide matrix. Similarly, a threshold should be reached at the bacterial cell surface in order to remove the Mg$^{2+}$ from the membrane gate to let the tobramycin in. Our experiments show that at the same current density in which we obtained total kill in the presence of antibiotics, there is still some killing of the biofilm in the absence of antibiotics; this also supports the theory by showing that at that specific current density, some cell membranes may be ruptured and certain cells would be killed.

Our results also show that the polarity of the system with respect to the nutrients flow makes little difference in the killing, but it is interesting to note that the pH in the upstream experiments changes to very acidic while it becomes very alkaline when the current is applied against the nutrient flow. The extreme change to acidic or alkaline in the pH of the fluid does not seem to affect the total killing of the bacterial biofilm. Our experiments show that this change in the pH is due to changes in the biofilm and not to electrolysis. The results of our polarity experiments also support the new theory, because the biofilm does not “see” the polarity of the electrical system.

The serial chambers experiments show that the killing effect was observed only when both the current density and the antibiotic were present. Therefore, a “pretreatment” of the antibiotic does not seem to be a viable hypothesis. This series of experiments also supports our new theory because the current must go through the biofilm in order to remove the cations in the exopolysaccharide and the cell membrane.
The angle of incidence between the electrical field and the biofilm appears to have an effect. It seems that some type of an acute angle is necessary for maximum killing. It is interesting to note that in Costerton’s experiments using the MRD the electrical field is perpendicular to the biofilm, but the biofilm was grown on the electrode. This was also the case for Jass’s experiments, but in her experiments the biofilm was grown separate from the electrodes. The results from those experiments are quite different from each other (see Table 47). The angle of the E field incidence alters the chemical and biological morphology of the system. The actual travel of the current through the biofilm may be enhanced if the correct angle is approached.

Our experiments using E-fields alone show no increase in killing. This result supports our new theory because a current is necessary in order to break the chemical bonds in the exopolysaccharide chain and in the cell membrane.

Recent information published by Dr. Jass and Dr. Lappin-Scott, the British team, show that the electrical current can enhance the activity against biofilms of only those antibiotics that are effective against the planktonic cells of the organism that form the biofilm [48]. This new information also supports our new theory because once the exopolysaccharide chains are broken, the antibiotic will act against the cells in a similar form that it would act against planktonic cells.

The experiments in our laboratories are the first to be done in this field of study that use pure DC low level current densities. Costerton’s group used a frequency of .0156
cycles/second (Hertz), while Jass’s group used a frequency of .0313 cycles/sec (Hertz).

This may be a possible reason for the difference in results reported by both researchers.

This is also the first time work has been done using AC induced currents.

Preliminary work using AC fields shows close to 2 log of increased killing. Even though this is not as much as the 6 logs obtained by using DC fields, it is still a very valuable increase in the killing of biofilm bacteria.
CONCLUSION

The understanding of biofilms and how they function has increased tremendously in the last decade. Now we know that they form "cities" in order to help each other and not only survive but flourish. New tools like mathematical models and confocal microscopy are helping us to elucidate these mechanisms. The understanding of these mechanisms in order to be able to control microbial biofilm infections in the medical field, and to control biofilm problems in industry is extremely important.

It is now understood that antimicrobial agents do penetrate the biofilm through the channels and other chemical venues. The biocides may react slowly with the exopolysaccharide matrix but this is not enough to be the sole cause of the added resistance. The cells, deep inside the microcolonies, do change their physiology due to their location in their environment. None of these theories by themself explain the whole process.

As part of this thesis, we have presented a possible mechanism for the killing of biofilm using antibiotics and a low level electrical current. From the results of our experiments, the new theory explained here, and the understanding that all living organism are electrical in nature, it can be postulated that this effect can be applied with similar results to other living organism and systems. It is also probable that the exact value of the current density needed to obtain maximum results may vary from system to system.

The possibilities for engineering applications of this new technology are many and widely applicable in the medical field as well as in industry. This technology will open a new age in the treatment of infections in the medical environment.
RECOMMENDATIONS

- Extend this work to other organisms and antimicrobial agents combinations.
- Establish a time dose response.
- Establish the lowest combination of antimicrobial concentration, current density and time for specific systems.
- Expand this work using AC induced current.
- Investigate the possibility of membrane rupture as the cause for biofilm killing using microbiological techniques.
- Extend this work into engineering practical solutions for known medical problems.
- Do a chemical analysis of the bulk fluid.
- Develop a mathematical model of the system.
- Expand the understanding of the change in pH.
REFERENCES CITED


APPENDICES
APPENDIX A
Calculations
$I = \int_0^s J ds$

$I = \int_0^s J dx dy$

$J = I / A$

where:
- Area ($A$) in cm²
- Current ($I$) in mA

Calculations:

<table>
<thead>
<tr>
<th>Box I</th>
<th>Box III</th>
<th>Box IV</th>
<th>Box V</th>
</tr>
</thead>
<tbody>
<tr>
<td>J = $\frac{2}{407}$ mA mm²</td>
<td>J = $\frac{2}{510}$ mA mm²</td>
<td>J = $\frac{2}{544}$ mA mm²</td>
<td>J = $\frac{2}{544}$ mA mm²</td>
</tr>
<tr>
<td>J = .4914 mA cm²</td>
<td>J = .3922 mA cm²</td>
<td>J = .3676 mA cm²</td>
<td>J = .3676 mA cm²</td>
</tr>
</tbody>
</table>

Conductivity Calculations

Box I
- Internal measurements:
  - $l$ - 78 mm
  - $w$ - 14.8 mm
  - $d$ - 27.5 mm

<table>
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<tr>
<th>VOLTS</th>
<th>RUN # 1 mA</th>
<th>RUN # 2 mA</th>
<th>AVE. Amps</th>
<th>R ohms</th>
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<td>2</td>
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<td>3</td>
<td>1.954</td>
<td>2.14</td>
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<tr>
<td>4</td>
<td>4.22</td>
<td>4.43</td>
<td>4.33E-02</td>
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<td>5</td>
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<tr>
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<td>72</td>
<td>7.28E-01</td>
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</tbody>
</table>

AVE. 1.10E+04 1.1K ohms

Area $14.8 \times 27.5 = 407$ mm²
Resistivity 5.76E+04 ohm-meter
Conductivity 1.74E-03 1/ohm-meter

Current Density (J)
Box I  \( J = 0.491400 \) mA/cm²
Box III  \( J = 0.392156 \) mA/cm²
Box IV  \( J = 0.367647 \) mA/cm²
Box v  \( J = 0.367647 \) mA/cm²

Conductivity (\( \sigma \)) \( \sigma = 1.74E-03 \) 1/ohm-meter

Power Density (\( w_p \)) \( w_p = I^2R = 0.0044 \) ohms
where \( I = 2 \) mA and \( R = 1.1 \) K ohms

\( E - Field (E) \) \( E^2 = w_p/\sigma \) 2.53 \( E = 1.59 \) V/m
APPENDIX B
Data by Groups
Control vs Positive Control Comparison
<table>
<thead>
<tr>
<th>Exp. #</th>
<th>CONTRO</th>
<th>P.C.</th>
<th>log(C/PC)</th>
</tr>
</thead>
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</tr>
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Average: 3.73E+09, 1.31E+09, 0.45, 2.51E+08, 1.25E+09, -0.70

Control - Antibiotics / No Fields
Positive Control - No Antibiotics / No Fields
Electrode Material Data
### OVERALL SUMMARY # 3

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### SUMMARY OF EXPERIMENTS # 7

| BACTERIA TYPE: Pseudomonas aeruginosa | FIELD TYPE: DC |
| BOX MODEL: III | SLIDE: STRAIGHT |
| ELECTRODE TYPE: WIRE | ELECTRODE MATERIAL: PLATINUM |
| POLARITY: UP STREAM | CURRENT: 1 mA |

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### SUMMARY OF EXPERIMENTS # 8

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| BOX MODEL: III | SLIDE: DIAGONAL |
| ELECTRODE TYPE: WIRE | ELECTRODE MATERIAL: PLATINUM |
| POLARITY: UP STREAM | CURRENT: 1 mA |

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**Slide Location / Electrode Material**

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### OVERALL SUMMARY # 10

**Polarity / Serial Chambers**

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### OVERALL SUMMARY # 11

**Dose Response with Antibiotic**

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Dose Response with Antibiotics Data
### OVERALL SUMMARY # 16

**Dose Response with Antibiotic**

**BACTERIA TYPE:** Pseudomonas aeruginosa  
**BOX MODEL:** VII  
**SLIDE:** STRAIGHT  
**CONTROL:** YES  
**ELECTRODE TYPE:** WIRES  
**POLARITY:** UP STREAM  
**FIELD TYPE:** DC  
**CURRENT:**

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### SUMMARY OF EXPERIMENTS # 45

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### OVERALL SUMMARY #1

**Dose Response with Antibiotic**

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| 1st SET: PC                          |                  |
| 2.10E+09                             | 3.70E+09         |
| 3.70E+09                             | 9.50E+08         |
| 9.50E+08                             | 1.70E+09         |
| 1st SET: E                           |                  |
| 2.58E+06                             | 1.43E+03         |
| 1.43E+03                             | 5.00E+02         |
| 5.00E+02                             | 5.00E+02         |
| E/PC                                 |                  |
| 0.001229                             | 3.86E-07         |
| 5.26E-07                             | 2.94E-07         |
| log (E/PC)                           |                  |
| 2.91                                 | 6.41            |
| 6.28                                 | 6.53            |

| 2nd SET: PC                          |                  |
| 8.70E+06                             | 1.50E+09         |
| 1.50E+09                             | 7.50E+08         |
| 7.50E+08                             | 1.70E+09         |
| 2nd SET: E                           |                  |
| 3.16E+06                             | 1.13E+04         |
| 1.13E+04                             | 5.00E+02         |
| E/PC                                 |                  |
| 0.363218                             | 7.15E-06         |
| log (E/PC)                           |                  |
| 0.44                                 | 5.15            |

| 3rd SET: PC                          |                  |
| 1.19E+09                             |                  |
| 1.19E+09                             |                  |
| 1.19E+09                             |                  |
| 3rd SET: E                           |                  |
| 1.33E+03                             |                  |
| log (E/PC)                           |                  |
| 1.12E-06                             |                  |
| 1.12E-06                             |                  |

| 4th SET: PC                          |                  |
| 1.85E+09                             |                  |
| 1.85E+09                             |                  |
| 1.85E+09                             |                  |
| 4th SET: E                           |                  |
| 5.00E+02                             |                  |
| E/PC                                 |                  |
| 2.7E-07                              |                  |
| log (E/PC)                           |                  |
| 6.57                                 |                  |

| AVE. SET: PC                         |                  |
| 1.05E+09                             | 2.60E+09         |
| 2.60E+09                             | 9.50E+08         |
| 9.50E+08                             | 1.70E+09         |
| AVE. SET: E                           |                  |
| 2.87E+06                             | 2.43E+03         |
| 2.43E+03                             | 5.00E+02         |
| 5.00E+02                             | 5.00E+02         |
| E/PC                                 |                  |
| 0.002733                             | 9.35E-07         |
| 5.26E-07                             | 2.94E-07         |
| log (E/PC)                           |                  |
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| 6.28                                 | 6.53            |
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**SUMMARY OF EXPERIMENTS # 3**

- **BACTERIA TYPE:** Pseudomonas aeruginosa
- **FIELD TYPE:** DC
- **CURRENT:** 7.5 mA
- **BOX MODEL:** I
- **SLIDE:** STRAIGHT
- **CONTROLLER:** NONE
- **ELECTRODE TYPE:** WIRE
- **ELECTRODE MATERIAL:** PLATINUM
- **POLARITY:** UP STREAM
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**Log (E/PC) Values**

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### SUMMARY OF EXPERIMENTS # 36

| BACTERIA TYPE: Pseudomonas aeruginosa | FIELD TYPE: DC | CURRENT: 1.5 mA |
| BOX MODEL: VII | SLIDE: STRAIGHT | CONTROLLER: YES |
| ELECTRODE TYPE: WIRE | ELECTRODE MATERIAL: SS |
| POLARITY: UP STREAM | ANTIBIOTICS: NONE |
| ORIGINAL COUNT | PLANTONIC | POSITIVE CONTROL | CONTROL | EXPERIMENT | E/PC | log (E/PC) |
| #30 | #35 | #36 | # | AVE. | DECREASES |
| 8.10E+09 | 8.20E+09 | 1.14E+10 | 9.23E+09 |
| 4.90E+05 | 1.18E+05 | 1.15E+06 | 5.86E+05 |
| 4.50E+08 | 5.40E+08 | 1.78E+09 | 9.23E+08 |
| 8.40E+07 | 7.00E+06 | 1.28E+07 | 3.46E+07 |
| 2.10E+07 | 1.01E+08 | 9.50E+07 | 7.23E+07 | -1.11 |
| 0.046667 | 0.187037 | 0.053371 | 0.078339 |
| 1.33 | 0.73 | 1.27 | 1.11 |

### SUMMARY OF EXPERIMENTS # 40

| BACTERIA TYPE: Pseudomonas aeruginosa | FIELD TYPE: DC | CURRENT: 2 mA |
| BOX MODEL: VII | SLIDE: STRAIGHT | CONTROLLER: YES |
| ELECTRODE TYPE: WIRE | ELECTRODE MATERIAL: SS |
| POLARITY: UP STREAM | ANTIBIOTICS: NONE |
| ORIGINAL COUNT | PLANTONIC | POSITIVE CONTROL | CONTROL | EXPERIMENT | E/PC | log (E/PC) |
| #32 | #34 | #36 | # | AVE. | DECREASES |
| 2.57E+09 | 8.70E+09 | 1.14E+10 | 7.56E+09 |
| 1.50E+03 | 1.83E+05 | 1.15E+06 | 4.45E+05 |
| 7.50E+08 | 1.51E+07 | 1.78E+09 | 8.48E+08 |
| 1.48E+08 | 6.60E+06 | 1.28E+07 | 5.68E+07 |
| 5.80E+06 | 1.11E+06 | 4.20E+07 | 1.63E+07 | -1.72 |
| 0.007733 | 0.07351 | 0.023596 | 0.019217 |
| 2.11 | 1.13 | 1.63 | 1.72 |

### SUMMARY OF EXPERIMENTS # 41

| BACTERIA TYPE: Pseudomonas aeruginosa | FIELD TYPE: DC | CURRENT: 3 mA |
| BOX MODEL: VII | SLIDE: STRAIGHT | CONTROLLER: YES |
| ELECTRODE TYPE: WIRE | ELECTRODE MATERIAL: SS |
| POLARITY: UP STREAM | ANTIBIOTICS: NONE |
| ORIGINAL COUNT | PLANTONIC | POSITIVE CONTROL | CONTROL | EXPERIMENT | E/PC | log (E/PC) |
| #32 | #35 | #36 | #37 | # | AVE. | DECREASES |
| 2.57E+09 | 8.20E+09 | 1.14E+10 | 2.69E+09 | 6.22E+09 |
| 1.50E+03 | 1.18E+05 | 1.15E+06 | 5.50E+02 | 3.18E+05 |
| 7.50E+08 | 5.40E+08 | 1.78E+09 | 5.60E+08 | 9.08E+08 |
| 1.48E+08 | 7.00E+06 | 1.28E+07 | 2.30E+06 | 4.25E+07 |
| 1.55E+08 | 3.40E+07 | 5.00E+02 | 1.10E+08 | 7.48E+07 | -1.08 |
| 0.206667 | 0.062963 | 2.81E-07 | 0.196429 | 0.082369 |
| 0.68 | 1.20 | 6.55 | 0.71 | 1.08 |
## OVERALL SUMMARY #7

**Dose Response without Antibiotic / Slide Location**

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### OVERALL SUMMARY # 8

Dose Response without Antibiotic

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Polarity Data
OVERALL SUMMARY # 17

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SUMMARY OF EXPERIMENTS # 46

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SUMMARY OF EXPERIMENTS # 47

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**E-Field / Polarity**

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### OVERALL SUMMARY #10

**Polarity / Serial Chambers**

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### SUMMARY OF EXPERIMENTS #10

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| ELECTRODE TYPE: WIRE |
| ELECTRODE MATERIAL: S.S. |
| POLARITY: UP STREAM |

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Slide Location Data
OVERALL SUMMARY #7

Dose Response without Antibiotic / Slide Location

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| log (E/PC) | 0.88 | 5.13 | 0.10 |

SUMMARY OF EXPERIMENTS # 20

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### OVERALL SUMMARY # 2

**Slide Location**

**BACTERIA TYPE:** Pseudomonas aeruginosa  
**FIELD TYPE:** DC

**BOX MODEL:** II  
**SLIDE:** STRAIGHT  
**CONTROLLER:** NONE

**ELECTRODE TYPE:** PLATES  
**ELECTRODE MATERIAL:** S.S.

**POLARITY:** UP STREAM  
**CURRENT**

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### SUMMARY OF EXPERIMENTS # 5

**BACTERIA TYPE:** Pseudomonas aeruginosa  
**FIELD TYPE:** DC

**BOX MODEL:** II  
**SLIDE:** STRAIGHT  
**CONTROLLER:** NONE

**ELECTRODE TYPE:** PLATE  
**ELECTRODE MATERIAL:** S.S.

**POLARITY:** UP STREAM  
**CURRENT:** 1 mA

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**FIELD TYPE:** DC

**BOX MODEL:** II  
**SLIDE:** STRAIGHT  
**CONTROLLER:** NONE

**ELECTRODE TYPE:** PLATE  
**ELECTRODE MATERIAL:** S.S.

**POLARITY:** UP STREAM  
**CURRENT:** 3 mA

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### OVERALL SUMMARY # 4

<table>
<thead>
<tr>
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<tr>
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<tr>
<td><strong>SLIDE:</strong></td>
</tr>
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<td><strong>ELECTRODE TYPE:</strong> WIRE</td>
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<tr>
<td><strong>ORIGINAL COUNT</strong></td>
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<td>1.72E+05</td>
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<td>1.36E+09</td>
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<tr>
<td>1.69E+07</td>
<td>6.50E+07</td>
</tr>
<tr>
<td>6.30E+03</td>
<td>2.45E+06</td>
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<td>8.90E-06</td>
<td>1.80E-03</td>
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### SUMMARY OF EXPERIMENTS # 10

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<td></td>
</tr>
<tr>
<td><strong>SLIDE:</strong></td>
<td><strong>ELECTRODE MATERIAL:</strong> S.S.</td>
<td></td>
</tr>
<tr>
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<th>Control</th>
<th>Experiment</th>
<th>(E/PC)</th>
<th>(\log(E/PC))</th>
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<td>3.80E+09</td>
<td>1.72E+05</td>
<td>1.36E+09</td>
<td>6.50E+07</td>
<td>5.00E+02</td>
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### SUMMARY OF EXPERIMENTS # 11

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<td><strong>BOX MODEL:</strong> IV</td>
<td><strong>CONTROLLER:</strong> NONE</td>
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</tr>
<tr>
<td><strong>SLIDE:</strong></td>
<td><strong>ELECTRODE MATERIAL:</strong> S.S.</td>
<td></td>
</tr>
<tr>
<td><strong>ELECTRODE TYPE:</strong> WIRE</td>
<td><strong>POLARITY:</strong> UP STREAM</td>
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<table>
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<th>Plantonic</th>
<th>Positive Control</th>
<th>Control</th>
<th>Experiment</th>
<th>(E/PC)</th>
<th>(\log(E/PC))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.80E+09</td>
<td>1.72E+05</td>
<td>1.36E+09</td>
<td>6.50E+07</td>
<td>5.00E+02</td>
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Serial Chambers Data
### OVERALL SUMMARY # 10

**Polarity / Serial Chambers**

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<td>BOX MODEL: VI</td>
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<td>ELECTRODE TYPE: WIRE</td>
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<tr>
<td>POLARITY:</td>
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</tr>
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<td>ORIGINAL COUNT</td>
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<td>POSITIVE CONTROL</td>
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</tr>
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<td>CONTROL</td>
<td>4.80E+07</td>
<td>4.80E+07</td>
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<td>EXPERIMENT</td>
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### SUMMARY OF EXPERIMENTS # 27

<table>
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<td>SLIDE: STRAIGHT</td>
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<tr>
<td>ELECTRODE TYPE: WIRE</td>
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</tr>
<tr>
<td>POLARITY: UP STREAM</td>
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<table>
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<th># 25</th>
<th># 26</th>
<th># 27</th>
<th># 28</th>
<th># 29</th>
<th>AVE. DECREASES</th>
</tr>
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<tbody>
<tr>
<td>ORIGINAL COUNT</td>
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<td>3.10E+09</td>
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<td>6.20E+09</td>
<td>1.96E+09</td>
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<tr>
<td>PLANTONIC</td>
<td>1.11E+06</td>
<td>5.00E+02</td>
<td>2.80E+06</td>
<td>2.34E+05</td>
<td>1.66E+05</td>
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<tr>
<td>POSITIVE CONTROL</td>
<td>4.70E+08</td>
<td>7.50E+08</td>
<td>1.90E+09</td>
<td>2.10E+08</td>
<td>8.33E+08</td>
</tr>
<tr>
<td>CONTROL</td>
<td>5.00E+06</td>
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<td>2.20E+07</td>
<td>2.00E+08</td>
<td>1.03E+07</td>
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<td>EXPERIMENT</td>
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<td>1.61E+06</td>
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<td>VALID EXPERIMENTS</td>
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<td>E/PC</td>
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### SUMMARY OF EXPERIMENTS # 28

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<tr>
<td>BOX MODEL: VI</td>
<td>SLIDE: STRAIGHT</td>
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</tr>
<tr>
<td>ELECTRODE TYPE: WIRE</td>
<td>ELECTRODE MATERIAL: S.S.</td>
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</tr>
<tr>
<td>POLARITY: DOWN STREAM</td>
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</tbody>
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<table>
<thead>
<tr>
<th># 25</th>
<th># 26</th>
<th># 27</th>
<th># 28</th>
<th># 29</th>
<th>AVE. DECREASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORIGINAL COUNT</td>
<td>7.00E+09</td>
<td>3.10E+09</td>
<td>3.80E+09</td>
<td>6.20E+09</td>
<td>1.96E+09</td>
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<td>PLANTONIC</td>
<td>1.11E+06</td>
<td>5.00E+02</td>
<td>2.80E+06</td>
<td>2.34E+05</td>
<td>1.66E+05</td>
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<tr>
<td>POSITIVE CONTROL</td>
<td>4.70E+08</td>
<td>7.50E+08</td>
<td>1.90E+09</td>
<td>2.10E+08</td>
<td>8.33E+08</td>
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<tr>
<td>CONTROL</td>
<td>5.00E+06</td>
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<td>2.20E+07</td>
<td>2.00E+08</td>
<td>1.03E+07</td>
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<tr>
<td>EXPERIMENT</td>
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<td>2.80E+06</td>
<td>1.31E+07</td>
<td>5.00E+07</td>
<td>2.90E+05</td>
</tr>
<tr>
<td>VALID EXPERIMENTS</td>
<td>6.10E+05</td>
<td>2.80E+06</td>
<td>1.31E+07</td>
<td>5.00E+07</td>
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<td>E/PC</td>
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E-Field Data
### OVERALL SUMMARY #5

**E-Field / Dose Response**

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<td>BOX MODEL: IV</td>
<td>SLIDE: STRAIGHT</td>
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<tr>
<td>ELECTRODE TYPE: WIRE</td>
<td>ELECTRODE MATERIAL: S.S.</td>
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</tr>
<tr>
<td>POLARITY: UP STREAM</td>
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<th>Positive Control</th>
<th>Control</th>
<th>Experiment</th>
<th>Valid Experiments</th>
<th>E/PC</th>
<th>log (E/PC)</th>
</tr>
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<tbody>
<tr>
<td>5.4 V</td>
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</tr>
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<td>10.8 V</td>
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<td>2.32E+07</td>
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<td>3.60E+07</td>
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### SUMMARY OF EXPERIMENTS #15

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<tr>
<td>ELECTRODE TYPE: WIRE</td>
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<td>POLARITY: UP STREAM</td>
<td>ELECTRODE LOCATION: OUTSIDE</td>
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<th>Positive Control</th>
<th>Control</th>
<th>Experiment</th>
<th>Valid Experiments</th>
<th>E/PC</th>
<th>log (E/PC)</th>
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### SUMMARY OF EXPERIMENTS #16

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<td>CONTROLLER: NONE</td>
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<th>Positive Control</th>
<th>Control</th>
<th>Experiment</th>
<th>Valid Experiments</th>
<th>E/PC</th>
<th>log (E/PC)</th>
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</thead>
<tbody>
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<td>6.70E+08</td>
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### OVERALL SUMMARY # 6

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<tr>
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<td>SLIDE: STRAIGHT</td>
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<tr>
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<td>ELECTRODE MATERIAL: S.S.</td>
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<tr>
<td>PLANTONIC</td>
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<tr>
<td>POSITIVE CONTROL</td>
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<tr>
<td>CONTROL</td>
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<td>EXPERIMENT</td>
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<td>VALID EXPERIMENTS</td>
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### SUMMARY OF EXPERIMENTS # 17

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<tr>
<td>ELECTRODE TYPE: WIRE</td>
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</tr>
<tr>
<td>ORIGINAL COUNT</td>
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<tr>
<td>PLANTONIC</td>
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<tr>
<td>POSITIVE CONTROL</td>
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<tr>
<td>CONTROL</td>
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<td>EXPERIMENT</td>
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<tr>
<td>VALID EXPERIMENTS</td>
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<tr>
<td>E/PC</td>
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<td>log (E/PC)</td>
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### SUMMARY OF EXPERIMENTS # 18

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<td>ELECTRODE TYPE: WIRE</td>
</tr>
<tr>
<td>POLARITY: DOWN STREAM</td>
</tr>
<tr>
<td># 19</td>
</tr>
<tr>
<td>ORIGINAL COUNT</td>
</tr>
<tr>
<td>PLANTONIC</td>
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<tr>
<td>POSITIVE CONTROL</td>
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<tr>
<td>CONTROL</td>
</tr>
<tr>
<td>EXPERIMENT</td>
</tr>
<tr>
<td>VALID EXPERIMENTS</td>
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<tr>
<td>E/PC</td>
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<td>log (E/PC)</td>
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Buffer Concentration Data
## OVERALL SUMMARY # 18

**Buffer Concentration**

<table>
<thead>
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<th>ELECTRODE MATERIAL: S.S.</th>
<th>POLARITY: UP STREAM</th>
<th>CURRENT: 2 mA</th>
</tr>
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<td><strong>Type:</strong> Pseudomonas aeruginosa</td>
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<td>5.00E+09</td>
<td>7.35E+09</td>
<td>6.80E+09</td>
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<td><strong>PLANKTONIC</strong></td>
<td>7.00E+02</td>
<td>1.85E+03</td>
<td>3.50E+06</td>
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<tr>
<td><strong>POSITIVE CONTROL</strong></td>
<td>3.90E+08</td>
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## SUMMARY OF EXPERIMENTS # 52

**BACTERIA TYPE:** Pseudomonas aeruginosa

**FIELD TYPE:** DC

**BOX MODEL:** VII

**SLIDE:** STRAIGHT

**ELECTRODE TYPE:** WIRE

**ELECTRODE MATERIAL:** SS

**POLARITY:** UP STREAM

**BUFFER CONC.: .5B**

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## SUMMARY OF EXPERIMENTS # 62

**BACTERIA TYPE:** Pseudomonas aeruginosa

**FIELD TYPE:** DC

**BOX MODEL:** VII

**SLIDE:** STRAIGHT

**ELECTRODE TYPE:** WIRE

**ELECTRODE MATERIAL:** SS

**POLARITY:** UP STREAM

**BUFFER CONC.: 1.1B**

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### SUMMARY OF EXPERIMENTS # 55

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### OVERALL SUMMARY # 19

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### SUMMARY OF EXPERIMENTS # 57

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| **BACTERIA TYPE:** Pseudomonas aeruginosa | **FIELD TYPE:** DC |
| **BOX MODEL:** VII | **SLIDE:** STRAIGHT |
| **ELECTRODE TYPE:** WIRE | **ELECTRODE MATERIAL:** SS |
| **POLARITY:** UP STREAM | **BUFFER CONC.: .5B** |
| **CURRENT:** 0 mA |  |

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### SUMMARY OF EXPERIMENTS # 61

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| **BACTERIA TYPE:** Pseudomonas aeruginosa | **FIELD TYPE:** DC |
| **BOX MODEL:** VII | **SLIDE:** STRAIGHT |
| **ELECTRODE TYPE:** WIRE | **ELECTRODE MATERIAL:** SS |
| **POLARITY:** UP STREAM | **BUFFER CONC.: 1.1B** |
| **CURRENT:** 0 mA |  |

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APPENDIX C
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**FIELD TYPE:** DC, 1mA | **SET # 2** | **EXP. # 1** | **ANTIBIOTIC CONC:** 5 mg/l

**GROWTH TIME:** 17.5 Hours

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**FIELD TYPE:** DC, 1mA  
**GROWTH TIME:** 20 Hours  

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**FIELD TYPE:** DC  **SET # 2**  **EXP. # 7**  **ANTIBIOTIC CONC:** 5 mg/l

**GROWTH TIME:** 17 Hours  **I:** 7.5 mA  **V:** v

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| 1.00E+05 | 26 | 32 | 31 | 33 | 22 | 22 | 26 | 26 | 23 | 32 | 2.73E+06 | 2.73E+08 |
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**FIELD TYPE:** DC  **SET # 2**  **EXP. # 9**  **ANTIBIOTIC CONC:** 5 mg/l

**GROWTH TIME:** 17 Hours  **I:** .5 mA  **V:**

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**ANTIBIOTIC CONC: 5 mg/l**

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**FIELD TYPE:** DC  |  **SET # 2**  |  **EXP. # 12**  |  **ANTIBIOTIC CONC:** 5 mg/l  
**GROWTH TIME:** 17 Hours  |  I: 3 mA  |  V:  n  

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|          | 1.00E+05 | 1 | 6 | 3 | 2 | 2 | 3 | 9 | 8 | 4  | 4.40E+05 | 4.40E+07 |
|          | 1.00E+01 | 0.5| 0.5| 0.5| 0.5| 0.5| 0.5| 0.5| 0.5| 0.5| 5.00E+00 | 5.00E+02 |
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DATE: 05-02-91

BACTERIA TYPE: PSEUDOMONAS AERUGINOSA

FIELD TYPE: DC

GROWTH TIME: 17 Hours

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**NEW BOX-DIAG.-SS PLATES**

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## BACTERIA TYPE: PSEUDOMONAS AERUGINOSA

**Field Type:** DC

**Set # 2**  
**Exp. # 16**  
**Antibiotic Conc:** 5 mg/l

### Growth Time: 17 Hours  
**I:** 1 mA  
**V:** v

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### Parameters:

- DATE: 05-09-91
- **Set # 2**
- **Exp. # 16**
- **Antibiotic Conc:** 5 mg/l
- **Growth Time:** 17 Hours
- **I:** 1 mA
- **V:** v
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**ANTIBIOTIC CONC:** 5 mg/l

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**BACTERIA TYPE:** PSEUDOMONAS AERUGINOSA  
**FIELD TYPE:** DC  
**GROWTH TIME:** 18 Hours  
**ANTIBIOTIC CONC:** 5 mg/l

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**Antibiotic/Plantonic**

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**Positive Control (no anti/ no field)**

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**Control (anti/no field)**

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**2C - NO ANTI/FIELD - 4 mA**

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**BACTERIA TYPE:** PSEUDOMONAS AERUGINOSA  
**FIELD TYPE:** DC  
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**EXP. # 24**  
**ANTIBIOTIC CONC:** 5 mg/l  

**GROWTH TIME:** 18 Hours  
**I:**  
**V:**  

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### ANTIBIOTIC/PLANTONIC

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### #1 - NO ANTI/FIELD - 1 mA

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<td>Control (anti/no field)</td>
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<td>#2 - Anti/Field - 2 mA - Reverse Polarity</td>
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<td>#2C - Anti/Field - .5 mA</td>
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**DATE:** 07-18-96  **BACTERIA TYPE:** PSEUDOMONAS AERUGINOSA  **FIELD TYPE:** DC  **GROWTH TIME:** 18 Hours  **SET #2**  **EXP. # 25**  **ANTIBIOTIC CONC:** 5 mg/l
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### ORIGINAL COUNT

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### ANTIBIOTIC/PLANTONIC

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### POSITIVE CONTROL (no anti/ no field)

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<th>AVE.</th>
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### CONTROL (anti/no field)

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### #1 - ANTI/FIELD - 2 mA

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### DILUTION | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | AVE. | cfu | ECREESE |
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### ANTIBIOTIC/PLANTONIC

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### POSITIVE CONTROL (no anti/no field)

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<tbody>
<tr>
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### #1 - ANTI/FIELD - 2 mA

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### #1C - ANTI/FIELD - w/controller - 1 mA

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### ANTIBIOTIC/PLANTONIC

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### CONTROL (anti/no field)

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### #1C - ANTI/FIELD -w/controller - 1 mA

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### POSITIVE CONTROL (no anti/ no field)

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<td>V:</td>
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**ORIGINAL COUNT**

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**ANTIBIOTIC/PLANTONIC**

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**POSITIVE CONTROL (no anti/ no field)**

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**CONTROL (anti/no field)**

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#1 - ANTI/FIELD - 1.5 mA w/controller

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# 2C - NO ANTI/FIELD - 1.5 mA w/controller

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# 3C - NO ANTI/FIELD - .5 mA w/controller

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**DATE:** 09-13-96  **BACTERIA TYPE:** PSEUDOMONAS AERUGINOSA  
**FIELD TYPE:** DC  
**GROWTH TIME:** 18 Hours  
**EXP. #** 31  
**ANTIBIOTIC CONC:** 5 mg/l  

### Original Count

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### Antibiotic/Plasmonic

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### Control (anti/no field)

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#### #1 - Antibiotic/Field - 2mA w/Controller

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**DATE: 09-19-96**
**BACTERIA TYPE: PSEUDOMONAS AERUGINOSA**
**FIELD TYPE: DC**
**SET # 2**
**EXP. # 32**
**ANTIBIOTIC CONC: 5 mg/l**

**GROWTH TIME: 18 Hours**

### ORIGINAL COUNT

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### ANTIBIOTIC/PLANTONIC

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### POSITIVE CONTROL (no anti/no field)

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### CONTROL (anti/no field)

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**#1 - ANTI/FIELD - 2 mA w/controller**

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<td>0.5</td>
<td>0.5</td>
<td>5.00E+00</td>
<td>5.00E+02</td>
<td>-6</td>
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</table>

**#2 - ANTI/FIELD - 3 mA w/controller**

<table>
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<tr>
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<th>10</th>
<th>AVE.</th>
<th>cfu</th>
<th>INCREASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00E+01</td>
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<td>5.00E+02</td>
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**#4 - ANTI/FIELD - 1 mA w/controller**

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<th>9</th>
<th>10</th>
<th>AVE.</th>
<th>cfu</th>
<th>INCREASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00E+01</td>
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</table>

**#2C - NO ANTI/FIELD - 2 mA w/controller**

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<th>INCREASE</th>
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<tbody>
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**#3C - NO ANTI/FIELD - 3 mA w/controller**

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<th>INCREASE</th>
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### ORIGINAL COUNT

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<th>10</th>
<th>AVE.</th>
<th>cfu</th>
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### ANTIBIOTIC/PLANTONIC

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<th>ECRICREASE</th>
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### POSITIVE CONTROL (no anti/ no field)

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<th>cfu</th>
<th>ECRICREASE</th>
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<tbody>
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### CONTROL (anti/no field)

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<th>AVE.</th>
<th>cfu</th>
<th>ECRICREASE</th>
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### #1 - ANTI/FIELD w/controller - .5 mA

<table>
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<th>9</th>
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<th>AVE.</th>
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<th>ECRICREASE</th>
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</thead>
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### #2 - ANTI/FIELD - w/controller - .25 mA

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<th>AVE.</th>
<th>cfu</th>
<th>ECRICREASE</th>
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</thead>
<tbody>
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### #4 - ANTI/FIELD - w/controller - .75 mA

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<tr>
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<th>4</th>
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<th>8</th>
<th>9</th>
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<th>AVE.</th>
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</thead>
<tbody>
<tr>
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### #3C - ANTI/FIELD - w/controller - 1 mA

<table>
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<tr>
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<th>9</th>
<th>10</th>
<th>AVE.</th>
<th>cfu</th>
<th>ECRICREASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00E+04</td>
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<td>16</td>
<td>13</td>
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### #2C - NO ANTI/FIELD - w/controller - 2 mA

<table>
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<th>AVE.</th>
<th>cfu</th>
<th>ECRICREASE</th>
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</thead>
<tbody>
<tr>
<td>1.00E+04</td>
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### Original Count

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<th>AVE. cfu</th>
<th>ECREASE</th>
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<tbody>
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<td>1.00E+07</td>
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### Antibiotic/Planktonic

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<th>10</th>
<th>AVE. cfu</th>
<th>ECREASE</th>
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<tbody>
<tr>
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### Positive Control (no anti/no field)

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<th>10</th>
<th>AVE. cfu</th>
<th>ECREASE</th>
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<tbody>
<tr>
<td>1.00E+04</td>
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### Control (anti/no field)

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<th>10</th>
<th>AVE. cfu</th>
<th>ECREASE</th>
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<tbody>
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### #1 - Anti/Field w/Controller - .5 mA

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<th>10</th>
<th>AVE. cfu</th>
<th>ECREASE</th>
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### #2 - Anti/Field - w/Controller - .25 mA

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<th>10</th>
<th>AVE. cfu</th>
<th>ECREASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00E+02</td>
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<td>1.00E+05</td>
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### #4 - Anti/Field - w/Controller - .75 mA

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<th>10</th>
<th>AVE. cfu</th>
<th>ECREASE</th>
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<tbody>
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### #3C - Anti/Field - w/Controller w/gasket - 1 mA

<table>
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<th>10</th>
<th>AVE. cfu</th>
<th>ECREASE</th>
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<tbody>
<tr>
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### #2C - No Anti/Field - w/Controller - 2 mA

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<th>10</th>
<th>AVE. cfu</th>
<th>ECREASE</th>
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</thead>
<tbody>
<tr>
<td>1.00E+03</td>
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FIELD TYPE: DC  SET # 2  EXP. # 35  ANTIBIOTIC CONC: 5 mg/l
GROWTH TIME: 18 Hours  I:  V: 

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ANTIBIOTIC/PLANTONIC

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POSITIVE CONTROL (no anti/ no field)

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CONTROL (anti/no field)

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#1 - NO ANTI/FIELD w/controller - 1 mA

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#2 - NO ANTI/FIELD - w/controller - 1.5 mA

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#2C - NO ANTI/FIELD - w/controller - .5 mA

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#3C - NO ANTI/FIELD - w/controller - 3 mA

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### ORIGINAL COUNT

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### ANTIBIOTIC/PLANTONIC

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### POSITIVE CONTROL (no anti/ no field)

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### CONTROL (anti/no field)

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### #1 - NO ANTI/FIELD w/controller - 3 mA

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### #2C - NO ANTI/FIELD - w/controller - 1 mA

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### #3C - NO ANTI/FIELD - w/controller - 1.5 mA

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**ORIGINAL COUNT**

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**ANTIBIOTIC/PLANTONIC**

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**POSITIVE CONTROL (no anti/no field)**

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**CONTROL (anti/no field)**

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**#1 - ANTI/FIELD 11.14 V/3 mA**

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<th>cfu</th>
<th>ECRIEASE</th>
</tr>
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**#2 - ANTI/FIELD w/ controller - 2 mA**

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<th>10</th>
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**#4 - ANTI/FIELD - w/ controller - 3 mA**

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<th>ECRIEASE</th>
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**# 2C - NO ANTI/FIELD - w/ controller - 3 mA**

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**# 3C - ANTI/FIELD - w/ controller - 2 mA - DOWN STREAM**

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### Field Type: DC

**Bacteria Type:** *Pseudomonas aeruginosa*

**Growth Time:** 18 Hours

<table>
<thead>
<tr>
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<th>Count 1</th>
<th>Count 2</th>
<th>Count 3</th>
<th>Count 4</th>
<th>Count 5</th>
<th>Count 6</th>
<th>Count 7</th>
<th>Count 8</th>
<th>Count 9</th>
<th>Count 10</th>
<th>Average</th>
<th>E.C.R. Increase</th>
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</thead>
<tbody>
<tr>
<td>1.00E+06</td>
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<td>24</td>
<td>33</td>
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<td>30</td>
<td>29</td>
<td>3.07E+07</td>
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</table>

| 1.00E+01 | 5        | 3        | 2        | 5        | 4        | 3        | 2        | 5        | 3.50E+01 | 3.50E+03 |

### Positive Control (No anti/no field)

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<th>Count 8</th>
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<th>Count 10</th>
<th>Average</th>
<th>E.C.R. Increase</th>
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### Control (anti/no field)

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<th>Count 4</th>
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<th>E.C.R. Increase</th>
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</thead>
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### #2 - Anti/Field - w/controller - 2 mA

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### #4 - Anti/Field - w/controller - 2 mA - Down Stream

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<th>E.C.R. Increase</th>
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### #2C - Anti/Field - w/controller - 3 mA

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<th>Count 10</th>
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### #3C - Anti/Field - w/controller - 3 mA - Down Stream

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<th>E.C.R. Increase</th>
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### Original Count

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<td>3</td>
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### Antibiotic/Plantonic

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### Positive Control (no anti/no field) - Slide

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<th>Ave.</th>
<th>Cfu</th>
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### Positive Control (no anti/no field) - Plantonic

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### Control (anti/no field) - Slide

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<th>10</th>
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<th>Ecrease</th>
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### Control (anti/no field) - Plantonic

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### #1 - Anti/Field - w/Controller -1 mA - Slide

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<th>Ecrease</th>
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### #1 - Anti/Field - w/Controller -1 mA - Down Stream

<table>
<thead>
<tr>
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<th>Cfu</th>
<th>Ecrease</th>
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<tbody>
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### #2 - Anti/Field - w/Controller - 1 mA - Plantonic - Down Stream

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<th>Cfu</th>
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### #4 - Anti/Field - w/Controller - 2 mA - Plantonic

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**Original Count**

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**Antibiotic/Planktonic**

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**Positive Control (no anti/no field) - Slide**

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**Control (anti/no field) - Slide**

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**#1 - Anti/No Field - 10% increased in buffer**

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<tbody>
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**#2 - Anti/Field - w/controller - 2 mA - 10% increased in buffer**

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**#4 - Anti/Field - w/controller - 2 mA - Regular buffer**

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<th>10</th>
<th>Ave.</th>
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<th>Increase</th>
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<tbody>
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**#2C - No Anti/Field - w/controller - 2 mA - 10% increased in buffer**

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<th>Increase</th>
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**#3C - No Anti/No Field - 10% increased in buffer**

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### Original Count

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### Antibiotic/Planktonic

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### Positive Control (no anti/ no field)

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### Control (anti/no field)

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### #1 - Anti/No Field - 5B Increased in buffer

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### #2 - Anti/Field - w/controller - 2 mA - 5B Increased in buffer

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### #4 - Anti/Field - w/controller - 2 mA - Regular buffer

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<th>10</th>
<th>Ave.</th>
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<tbody>
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### #2C

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### #3C

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### #3C

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**ORIGINAL #1 - ANTI/NO FIELD - 5B Increased in buffer**

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**ORIGINAL #2 - ANTI/FIELD - w/control - 2 mA - 5B Increased in buffer**

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**ORIGINAL #3 - ANTI/NO FIELD - Regular buffer**

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**ORIGINAL #4 - ANTI/FIELD - w/controller - 2 mA - Regular buffer**

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**Original Count**

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<th>10</th>
<th>Ave. CFU</th>
<th>Increase</th>
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**Antibiotic/Plantonic**

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**Positive Control (no anti/ no field)**

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**Control (anti/no field) (contaminated)**

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<th>10</th>
<th>Ave. CFU</th>
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**#1 - Anti - pH 4.25**

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**#2 - Anti - pH 4.25**

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**#3C - No Anti - pH 4.25**

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**Dilution**

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<th>10</th>
<th>Ave. CFU</th>
<th>Increase</th>
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**BACTERIA TYPE**: PSEUDOMONAS AERUGINOSA

**GROWTH TIME**: 18 Hours  
**FIELD TYPE**: DC  
**SET #**: 2  
**EXP. #**: 46  
**ANTIBIOTIC CONC**: 5 mg/l

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### Original Count

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### Antibiotic/Plasmonic

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### Positive Control (no anti/ no field)

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<th>DECREASE</th>
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### Control (anti/no field)

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<th>DECREASE</th>
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### #1 - Anti - pH 9

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### #2 - Anti - pH 9

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### #4 - Anti/Field - pH 9 - 2 mA

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<th>DECREASE</th>
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<tbody>
<tr>
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### #3C - Anti/Field - pH 9 - .5 mA

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### #2C - No Anti - pH 9

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<th>DECREASE</th>
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<tr>
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<td>22</td>
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**BACTERIA TYPE:** PSEUDOMONAS AERUGINOSA  
**FIELD TYPE:** DC  
**GROWTH TIME:** 18 Hours  
**SET # 2**  
**EXP. # 47**  
**ANTIBIOTIC CONC:** 5 mg/l

### ORIGINAL COUNT

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### ANTIBIOTIC/PLANTONIC

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### POSITIVE CONTROL (no anti/ no field)

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### CONTROL (anti/no field)

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### #1 - ANTI - 50% less B

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### #4 - ANTI/FIELD - RB - 2 mA

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<tr>
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### #3C - ANTI/FIELD - RB - .75 mA

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### ORIGINAL COUNT

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### ANTIBIOTIC/PLANTONIC

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### POSITIVE CONTROL (no anti/ no field)

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### CONTROL (anti/no field)

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### #1 - ANTI/FIELD - 50% less B - 2 mA

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### #4 - ANTI/FIELD - RB - 2 mA

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### #3C - ANTI/FIELD - 4B - 2 mA

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### #2C - ANTI/FIELD - 4B - 2 mA

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DATE: 01-30-96  BACTERIA TYPE: PSEUDOMONAS AERUGINOSA
FIELD TYPE: DC  SET # 2  EXP. # 49  ANTIBIOTIC CONC: 5 mg/l
GROWTH TIME: 18 Hours  I:  V:  V

ORIGINAL COUNT

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ANTIBIOTIC/PLANTONIC

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<th>AVE.</th>
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<th>ECREESE</th>
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<td>0.5</td>
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POSITIVE CONTROL (no anti/ no field)

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CONTROL (anti/no field) contaminated

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<th>ECREESE</th>
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#1 - ANTI/FIELD - 5B - 2 mA

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<th>10</th>
<th>AVE.</th>
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<th>ECREESE</th>
</tr>
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<tbody>
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#2 - ANTI/FIELD - 5B - 2 mA

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<th>10</th>
<th>AVE.</th>
<th>cfu</th>
<th>ECREESE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00E+02</td>
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<td>24</td>
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#4 - ANTI/FIELD - RB - .75 mA

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<th>10</th>
<th>AVE.</th>
<th>cfu</th>
<th>ECREESE</th>
</tr>
</thead>
<tbody>
<tr>
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#3C - ANTI/FIELD - RB - .25 mA

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<th>7</th>
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<th>10</th>
<th>AVE.</th>
<th>cfu</th>
<th>ECREESE</th>
</tr>
</thead>
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<tr>
<td>1.00E+02</td>
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#2C - NO ANTI/FIELD - RB - 2 mA

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<th>AVE.</th>
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<th>ECREESE</th>
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</table>
DATE: 02-06-96  BACTERIA TYPE: PSEUDOMONAS AERUGINOSA

FIELD TYPE: DC  SET # 2  EXP. # 50  ANTIBIOTIC CONC: 5 mg/l
GROWTH TIME: 18 Hours  l:  V: v

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<tbody>
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**ORIGINAL COUNT**

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**ANTIBIOTIC/PLANTONIC**

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**POSITIVE CONTROL (no anti/ no field)**

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<tr>
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**CONTROL (anti/no field)**

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**#1 - ANTI/FIELD - 3B - 2 mA**

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<th>3</th>
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**#2 - ANTI/FIELD - 3B - 2 mA**

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**#4 - ANTI/FIELD - 4B - 2 mA**

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**#3C - ANTI/FIELD - 2B - 2 mA**

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**#2C - NO ANTI/FIELD - RB - 2 mA**

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<tbody>
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<td>1.00E+06</td>
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APPENDIX D
Data by Groups
AC Coupled
# OVERALL SUMMARY # 1

<table>
<thead>
<tr>
<th>BACTERIA TYPE: Pseudomonas aeruginosa</th>
<th>FIELD TYPE: AC</th>
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<tbody>
<tr>
<td>BOX MODEL: I</td>
<td>SLIDE: STRAIGHT</td>
</tr>
<tr>
<td>TYPE of FIELD: Solenoid</td>
<td>FREQ.: 24 MHz</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>VOLTAGE</th>
<th>10 mV</th>
<th>40 mV</th>
<th>80 mV</th>
<th>100 mV</th>
<th>300 mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORIGINAL COUNT</td>
<td>2.00E+09</td>
<td>5.96E+09</td>
<td>1.99E+09</td>
<td>3.19E+09</td>
<td>3.66E+09</td>
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<tr>
<td>POSITIVE CONTROL</td>
<td>1.19E+09</td>
<td>4.73E+09</td>
<td>1.70E+09</td>
<td>1.58E+09</td>
<td>3.19E+09</td>
</tr>
<tr>
<td>CONTROL</td>
<td>4.10E+07</td>
<td>5.58E+08</td>
<td>1.06E+08</td>
<td>1.83E+09</td>
<td>1.40E+08</td>
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<tr>
<td>EXPERIMENT</td>
<td>2.76E+08</td>
<td>6.37E+07</td>
<td>1.25E+08</td>
<td>3.19E+09</td>
<td>8.17E+08</td>
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<tr>
<td>VALID EXPERIMENTS</td>
<td>2.76E+08</td>
<td>6.37E+07</td>
<td>1.25E+08</td>
<td>3.19E+09</td>
<td>8.17E+08</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>VOLTAGE</th>
<th>10 mV</th>
<th>40 mV</th>
<th>80 mV</th>
<th>100 mV</th>
<th>100 mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st SET: PC</td>
<td>1.19E+09</td>
<td>8.67E+09</td>
<td>1.70E+09</td>
<td>1.58E+09</td>
<td>1.85E+09</td>
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<tr>
<td>1st SET: E</td>
<td>2.76E+08</td>
<td>1.40E+07</td>
<td>1.25E+08</td>
<td>3.19E+09</td>
<td>4.70E+07</td>
</tr>
<tr>
<td>E/PC</td>
<td>0.231933</td>
<td>0.001615</td>
<td>0.073529</td>
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<td>0.025405</td>
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<tr>
<td>log (E/PC)</td>
<td>0.63</td>
<td>2.79</td>
<td>1.13</td>
<td>-0.31</td>
<td>1.60</td>
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<td>2nd SET: PC</td>
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<td>2nd SET: E</td>
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**EXP. # 3**  
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**FIELD TYPE:** AC  
**FREQ.:** 24 MHz  
**P. Volts:** 40 mV  
**TYPE of FIELD:** Solenoid

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**GROWTH TIME:** 17 Hours  
**FIELD TYPE:** AC  
**FREQ.:** 24 MHz  
**P. Volts:** 40 mV  
**EXP. #** 6  
**SET #** 2  
**ANTEBIOITIC CONC.:** 5 mg/l  
**TYPE of FIELD:** Solenoid

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**Bacteria Type:** Pseudomonas Aeruginosa  
**Growth Time:** 17 Hours  
**Set #2**  
**Exp. #7**  
**Antibiotic Conc.:** 5 mg/l  
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P. Volts: 300 mV  
TYPE of FIELD: Solenoid  
ANTIBIOTIC CONC: 5 mg/l

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**P. Volts:** 300 mV  
**TYPE of FIELD:** Solenoid

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| 1.00E+06 | 20 | 17 | 17 | 26 | 22 | 27 | 20 | 20 | 29 | 20 | 2.18E+07 | 2.00E+03 | 2.18E+09 |
| 1.00E+06 | 20 | 9  | 9  | 12 | 15 | 13 | 20 | 13 | 11 | 12 | 1.34E+07 | 1.20E+03 | 1.34E+09 |
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