

The Use of Bacterial Batteries to Generate Hydrogen Gas
by

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For My Father and Mother

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Abstract:

It was hypothesized that *Geobacter* present in untreated sewage would metabolize organic matter, and given appropriate graphite electrodes, produce an electric current that could further be used to produce hydrogen gas. Six bacterial batteries were constructed using 10-gallon aquaria, each including two graphite anodes (in the tank bottom) and two graphite cathodes (near the tank surface). A semi-porous Plexiglas barrier was inserted between the anodes and cathodes to create an anoxic environment for *Geobacter* on the anodes. The anodes served as the metabolic electron acceptor for the bacteria. Current generated traveled from anode to cathode and was monitored using ammeters. After allowing the bacterial batteries to equilibrate with no added *Geobacter*, no current was observed. Later, the battery cells were inoculated with dilute sediment slurry collected from another working battery made using riparian wetland soil. Injection of acetic acid into the cells caused increased current generation in all cells.

After initial tests, three experiments were performed using these batteries and included the addition of untreated sewage to test the efficacy of this system to generate current at the expense of the mineralization of organic matter in the sewage. Each experiment involved adding a new batch of raw sewage then measuring the currents produced, dissolved oxygen levels, pH levels, and percent organic matter within the solid sewage and in the water of the batteries. Additional tests were designed to determine the efficacy of this battery system to store energy generated as the sewage organic matter is mineralized via electrolysis of H₂O into H₂.

Implications of the study suggest that organic biomass and hydrogen can be seen as likely alternatives in creating new sources of energy from renewable resources.

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Introduction:

Purpose

This research sought to develop and test a method for generating an electrical current using a uniquely designed bacterial battery, and then to test the potential use of these batteries in the electrolytic production of hydrogen gas (H₂). The utilization of sewage sludge to produce electrical current in other bacterial batteries in Dr. Henry Spratt's lab suggests that raw sewage could act as an excellent carbon source for bacterial metabolism¹⁸. The bacterial battery assembly consisted of four graphite electrodes, two anodes and two cathodes, securely connected in a parallel circuit to ammeters. One variation in the experimental design focused on whether an increase in surface area on the electrodes allowed for a greater current production. The percent organic matter present in the raw sewage was also an important factor in determining the magnitude of current. One concern throughout the procedure was whether the Plexiglas partition succeeded as an efficient membrane in isolating an anoxic environment containing graphite anodes from a fully oxygenated environment containing graphite cathodes. Finally, the project sought to test whether the battery generated current could efficiently be stored in the form of hydrogen gas by means of the electrolysis of water into hydrogen and oxygen. The possibility of using organic waste as a basic source for electricity seems reasonable with the high-energy demands and waste problems evident worldwide^{7, 18, 20}. Developing an efficient bacterial battery system that could regularly act as a source of self-perpetuating power while simultaneously breaking down organic matter in human wastewater may be the

paradigm needed to create an inexhaustible and clean energy source for a sustainable future.

Global energy needs, dwindling fossil fuels, and inexhaustible alternatives

The need for alternative sources of energy and more efficient energy generation are pertinent in the generation of a sustainable society for the future. As industry and population continue to grow, a shift to an extended and more diversified use of electricity will occur. The transition from primitive agriculture to modern agriculture and modern industrialization has greatly contributed to this high dependency on electricity and other energy sources ¹. Recent global energy demands and consumption reached an approximate high of 25 terrawatts (TW) ($1\text{TW}=1\times 10^{12}\text{W}$) in 1995, with an annual energy requirement of 10^{21} Joules ². It is estimated that the worldwide power demand will even reach an amount as high as 100 TW globally ². By the year 2020, electricity use is predicted to increase by 75 % ³. Currently, humans depend mainly on fossil fuels, nuclear energy, and waterpower as the main sources of energy ⁴. The ultimate dependency on the earth's natural resources for energy has resulted in massive amounts of pollution, such as carbon dioxide (CO_2) emissions, and diminishing global energy reserves due to the use of these finite energy resources. This problem has been escalating since the industrial revolution with excess amounts of hazardous pollutants contaminating and destroying the earth's soil, water supply, and atmosphere. Many of these pollutants result from the excessive use of fossil fuels.

In order to produce a sustainable society for the future, clean, renewable energy sources need to be developed. The types of useable energy on earth derive from several non-renewable and renewable resources. In the future dwindling fossil fuel supply poses increasingly difficult problems related to transportation, residential expansion, industrial growth, commercial, and additional energy and fuel dependent sectors. For example, the methods for retrieving coal can result in extreme environmental problems. One coal mining technique, mountain top removal, can negatively affect water quality and upset other aspects of local ecosystems. According to the Energy Information Administration (EIA), world marketed energy consumption by the year 2030 is expected to increase by 57%⁵. Approximately 90% of the world's energy consumption comes from the earth's non-renewable fossil fuels such as natural gas, oil, and coal². Today, natural gas supplies about 21% of energy used mostly to produce heat and steam, and coal accounts for about 26% of commercial energy⁴. Petroleum, nuclear power, and waterpower mainly supply the remaining amount of energy used here on earth. Within the next 30 years, the transportation sector will continue to heavily rely on use of petroleum and other liquid fuels. In 2004, this sector's total demand for liquid fuel (or petroleum) was at 58% of energy used and the demand is predicted to increase to 63% within the next 20 years⁵. It is predicted that the main petroleum supply will continue to diminish in abundance until to the year 2060, where the reliance on natural gas, coal, hydroelectric, and nuclear energy will then increase to compensate for the diminishing petroleum resource². At present time, society operates and depends on

an oil supply that is predicted to be depleted and increasingly more costly within the next 100 years. With the prolonged dependency on the earth's limited oil and other fossil fuel supply, pollution and global warming are seen as potential deleterious effects to the environment ². With the decline in the availability of the most widely used source of petroleum, humans need new sources of renewable energy.

The EIA explains that consumption of coal and natural gas, two other non-renewable energy sources, is projected to increase annually at a rate of 2.8% and 3.3%, respectively, from 2004 to 2030. Today, 80% of worldwide electricity power generation depends on these two fossil fuels ⁵. In 2004, the residential sector of worldwide energy consumed about 11% of global energy. The average monthly residential electricity consumption was 938 kilowatt hours (kWh) ⁵. As suburban areas continue to develop and as individual houses continue to grow in size and in number of occupants utilizing electricity within the home, the amount of power needed within these residences will also expand. It is expected in 2025 that natural gas will be surpassed by electricity as the prominent household energy provider ⁵. In addition, commercial sectors, including industrial and agricultural services, will continue to consume worldwide energy. Inevitably, energy demand will continue to increase with continuing developments, advances in technology, and economic growth ⁵.

Ultimately, new inexhaustible energy sources, such as bacterial batteries, which pose no harm to the environment, need to be further developed in order to be made commercially viable to society. With increasing dependence on the use of

energy, it is pertinent to be on route toward other sources of energy that could generally conserve the earth's non-renewable resources. Through the utilization of natural, everyday human waste, bacterial batteries may offer practical applications in a variety of needed areas, such as industrial manufacturing, farms, households, and wastewater treatment plants. Organic biomass and hydrogen are seen as likely options in creating new sources of energy from renewable resources.

Binary fission nuclear power plants

Though coal remains the primary source for electricity generation, nuclear power plants, oil, and natural gas have also been sources for electricity since the 1970's. Since world oil prices have increased, worldwide electricity generation using oil has become much more costly. Concern regarding potential dwindling fossil fuel supplies has recently given nuclear fission plants reinvigorated consideration ⁴. Nuclear fission facilities utilize the energy within atoms to generate heat, which is then used in a manner similar to that used by typical fossil fuel plants to produce electricity ⁶. Conventional power plants burn fossil fuels such as oil or coal to generate steam to rotate turbines and generate electricity.

In efforts to preserve the limited fossil fuel resources, nuclear fission plants can generate electricity in a similar manner using uranium (²³⁵U) to produce heat instead of coal or oil ⁶. Though uranium reserves are also exhaustible, generating electricity in a nuclear power requires significantly smaller amounts of uranium in comparison to the amount of fossil fuels needed to power an electricity plant. Where

conventional power plants may require about three million pounds of coal to produce enough energy to fulfill its electricity demand, nuclear fission can release more energy using only one pound of uranium ⁴. However, it is predicted that total worldwide uranium resources are capable of generating approximately 100 TW/yr (e.g. 1.000¹¹ kW) of electricity using nuclear fission technology. Thus, within the next decade, it is estimated that the finite uranium resource will be depleted if conventional nuclear fission methods continue to supply 10 TW of power per year.

Projections for electricity generated by nuclear power plants suggest an increase of 1.3 % annually. Such a rate of increase in these plants brings up concerns about the amounts of radioactive wastes generated through their use ⁵. Many European countries are planning to prevent the building and future use of nuclear plants, whereas larger and more populated countries such as India and China plan to rely more heavily on them with yearly rates of increasing electricity generation reaching 7% to 9 % per year ^{3,5}. Countries planning on resorting on costly electricity sources may soon be able to invest in the environment friendly electricity that bacterial batteries can offer. In order provide for a sustainable future in terms of energy production, the need for a shift to renewable energy sources has become pertinent. Although sewage is conventionally viewed as simply a pollutant, it can now be more appropriately defined as wasted energy. Incorporating this sewage waste into bacterial batteries that in turn can produce electricity could potentially serve as an efficient renewable energy source.

Hydrogen generation via electrolysis

A shift toward a hydrogen-powered society has become another popular area of interest in the conquest of a sustainable society. Hydrogen can be produced from a variety of fossil fuels, biomass, nuclear energy, and numerous other renewable resources. In addition to its production from non-fossil fuel resources, it can help reduce the amount of global CO₂ emissions. Numerous electrolytic hydrogen production technologies and systems have already been developed and are continuing to be developed ²⁷. They exist in a wide range of hydrogen power output and sizes vary from powering a small household to a large fueling station ²⁷. The economic cost to produce smaller fuel cell systems powering households and small neighborhoods is feasible ²⁷.

The energetics of electrolysis is simple in that water molecules can be split into hydrogen at the cathode and oxygen at the anode inside an electrolytic cell ²⁷. An electrolytic cell requires a power source to cause a reaction between water and the electrodes to occur. With adequate amounts of electrical current passing through two electrodes in pure water, hydrogen can be produced. Today, three electrolysis technologies exist and are currently being used. One type of electrolysis system is referred to as Proton Exchange Membrane (PEM) or Solid Polymer Electrolyte. The PEMs allow for oxygen and hydrogen to be produced and remain separated in opposite chambers. Within this system, a solid ion conducting membrane serves as the electrolyte that generates hydrogen via the transport of protons from the anode side to the cathode side. The most common form of electrolysis is designed so that a

highly conductive aqueous solution of potassium hydroxide (KOH) serves as the alkaline electrolyzer. The electrolyzer can be designed as unipolar or bipolar. Both systems contain a membrane that separates the electrodes, allows the transfer of ions, and prevents gasses from mixing. The electrodes of a unipolar design form a parallel connection whereas the bipolar design connects the electrodes in a series to produce oxygen and hydrogen gases in two separate chambers.²⁷

Additional research is needed to further explore the cost/benefit ratios of using hydrogen produced via the electrolytic process. The effectiveness and cost of the electrolytic method combined with the principal cost of electricity plays a huge role in the efficacy of using electrical current to generate hydrogen. Using an inexpensive form of electricity is pertinent to improving the efficiency and applicability of electrolysis as an energy source. The cost of renewably produced hydrogen needs to be reduced to be competitive with traditional fuel sources. By harvesting the wasted energy in the organic component of sewage, hydrogen could be produced and stored for later use. The current project was aimed at testing an electrolytic system that potentially could use the current generated from bacterial batteries to create usable hydrogen.

Geobacter sulfurreducens

Through recent studies and comprehensive microbiological research, microbes capable of producing electricity given appropriate graphite electrodes have been discovered^{8, 11, 12}. Conventionally, bacteria living in anoxic environments metabolize

organic matter to gain electrons for the production of adenosine triphosphate (ATP)⁷. These microorganisms participating in metabolic pathways become energized by reducing a substrate, such as a concentrated sugar or organic waste, and send the electrons to a final electron acceptor^{8,9}. Sometimes bacteria require the assistance of a soluble mediator to enhance electron transfer⁹. Research suggests that these microorganisms living in their natural habitats significantly use this electron transfer to create enough energy for proper metabolism and healthy growth⁸.

Certain bacteria can easily survive and live in anaerobic sediment containing high concentrations of insoluble metals, such as Fe (III) or Mn (IV)^{8,10}. Scientists have found that the ability of these bacteria to use novel electron acceptors for their metabolism gives these cells a unique advantage⁸. Such microbes are often referred to as electricigens and belong to the family, *Geobacteraceae*. They have the capacity to oxidize organic substances to CO₂ while depositing electrons obtained from the oxidation on a graphite electrode (anode) when connected to another electrode known as the cathode, serving as the electron acceptor for anaerobic respiration^{8,11,12}. After reaching the cathode, oxygen and protons merge with the electrons to make water¹³. Additionally, the self-perpetuating activity of these microorganisms within a microbial fuel cell suggests a possibility for them to convert significant amounts of organic waste into electricity without the role of a mediator^{11,12}.

Derek Lovley, a microbiologist from the University of Massachusetts, Amherst, began studying the factors involved in anaerobic respiration among microorganisms during the late 1980's. He first noticed that high hydrogen levels in

aquatic sediment indicated the presence of ongoing redox reactions⁷. In 1998, several years after his discovery of *Geobacter sulfurreducens*, Lovley found that this species of bacteria, along with other iron-oxide reducing bacteria, could effectively produce and collect high concentrations of hydrogen from organic materials in the presence of abundant electron donors (acetate, lactate, etc) and restricted transfer to an electron acceptor^{13, 15}. Finally, in 2003, he discovered that *Geobacter sulfurreducens* growing directly on the anodes of bacterial batteries had full capacity of producing electricity within such areas of high organic concentration using electrodes as their terminal electron acceptor (TEA)^{7, 16, 17}.

Other studies have proven that the *Geobacteraceae* family of bacteria is not the only bacteria that can completely oxidize organic substances, such as acetate, through the constant transfer of electrons to electrodes¹⁶. Bacteria known as *Rhodospirillum rubrum* and *Shewanella putrefaciens* oxidize glucose and lactate, respectively, while using a graphite electrode as the TEA^{16, 19}. With evidence proving the efficacy of certain bacterial species to produce electricity in natural environments, scientists are well on the way to developing ways to utilize the global, abundant supply of organic biomass from municipal, cultivation, and industrial sources. With years of research, the emergence of bacterial batteries may finally offer a renewable, clean alternative to energy generation that society will require to sustain an electrical future once the fossil fuel supply becomes depleted; sooner if measures to curtail global warming lead to drastic measures to reduce fossil fuel use. The ability of these microorganisms to produce significant amounts of electricity

seems promising in an era largely dependent on the utilization of electricity.

Bacterial battery construction and operation

A bacterial battery, often referred to as a microbial fuel cell (MFC), provides electricity through the oxidation of organic fuels while transferring electrons to electrodes without any assistance from mediators. As explained earlier, bacterial batteries operate by means of bacterial metabolism of organic matter within sediment, human wastewater, and food wastewater using graphite electrodes as their TEAs²⁰. The efficacy of the system depends on the structure and mechanics of the battery and the specific type of organic matter being used¹⁷. A typical battery requires two graphite electrodes, an anode and a cathode, in order to generate electricity. The anode is embedded within the anaerobic portion of the battery cell containing the organic biomass, whereas the cathode resides in an overlying, fully oxygenated area^{17,20}. Some bacterial batteries may include multiple electrodes in anoxic and aerobic portions of the cell.

The construction of a single-chambered battery or a bi-chambered battery depends on the overall configured bacterial battery and relies on the inclusion of a semi-permeable membrane to separate aerobic from anaerobic portions of the cell. For example, many bacterial batteries have been found to require a proton-exchange membrane (PEM) or a cation-selective membrane that assists in channeling the protons in only one direction from the anode to the cathode^{11, 18, 19}. Furthermore, the membrane prevents oxygen from entering the anode chamber where it could inhibit

the metabolic activity of the bacteria ¹¹. In one particular study, researchers found that no membrane was needed in a single-chambered battery due to the natural separation of anoxic seafloor deposit and oxygenated seawater ²¹. However, the current research required the incorporation of a semi-porous Plexiglas barrier to create a two-chambered battery and to confine the dissolved oxygen in the upper cathode chamber.

Finally, electrical circuits connecting the anode and cathodes interrupt the flow of electrons to the cathode, which in turn enables the electricity to be run through an ammeter for current monitoring before being sent to the cathode ¹⁷. As long as a constant provision of organic fuel remains in supply, bacteria growing on or near the anodes can sufficiently survive off the little energy that is produced during respiration ¹¹.

Optimization and utilization of bacterial batteries

Though bacterial batteries cannot yet demonstrate power generation for large-scale applications, its current capabilities may provide enough energy needed locally for individual appliance and household electricity generation while simultaneously processing sewage and cleaning wastewater ¹¹. Studies illustrating the optimistic abilities of these bacterial batteries continue to increase as research in the field of microbial energy production continues to demonstrate realistic energy capabilities.

After discovering the initial electrical capabilities of members of the *Geobacteraceae* and their link to the use of organic matter, researchers focused on

ways in which bacteria may be useful in dealing with the problem of the large and constant supply of wastewater. Today, it is estimated that 1.5% of the electricity produced in the United States is used in wastewater treatment ⁷. Some sewage treatment plants are already utilizing an anaerobic treatment process to create biogases that can be used to generate a third of the available energy content possible from bacteria ¹⁸. Lovley's research showing the success of *Geobacter* in producing electricity from marine sediment has paved the way for him and other scientists to research ways in which these microorganisms can convert a variety of sources of organic matter, including human wastewater, into electricity ²¹.

Specific research has been aimed at generating alternative energy sources while simultaneously treating agricultural and industrial wastewater ¹⁸. With the large masses of wastewater constantly overflowing treatment plants worldwide, alternative energy sources supplied from bacteria could aid in sufficient treatment and electricity needed to run treatment plants. In-situ bioremediation becomes a plausible tool in which naturally living bacteria in subsurface wastewater areas could become a weapon in the battle against sewage contamination ²¹. Lovley (2001) found that benzene and other aromatic hydrocarbons, both abundant and harmful components in petroleum wastewater, could quickly be degraded by anaerobes in the *Geobacteraceae* group ²⁴. Together with aerobes, it seems possible for these anoxic bacteria to possibly remediate contaminated waters by speeding up the degradation process using increased amounts of sulfate or nitrate. Ultimately, he compared the benefits of using anoxic organisms, opposed to aerobic organisms, in bioremediation

of wastewaters ²⁴. Further studies suggest that the presence of *Geobacter* on an electrode placed in uranium-contaminated sediments can prevent the migration of harmful uranium by reducing U(VI) into U(IV) ²⁵. Lovley's extensive work on the use of MFCs shows promise in creating a self-sustaining bacterial battery that could concurrently treat waste while producing usable energy.

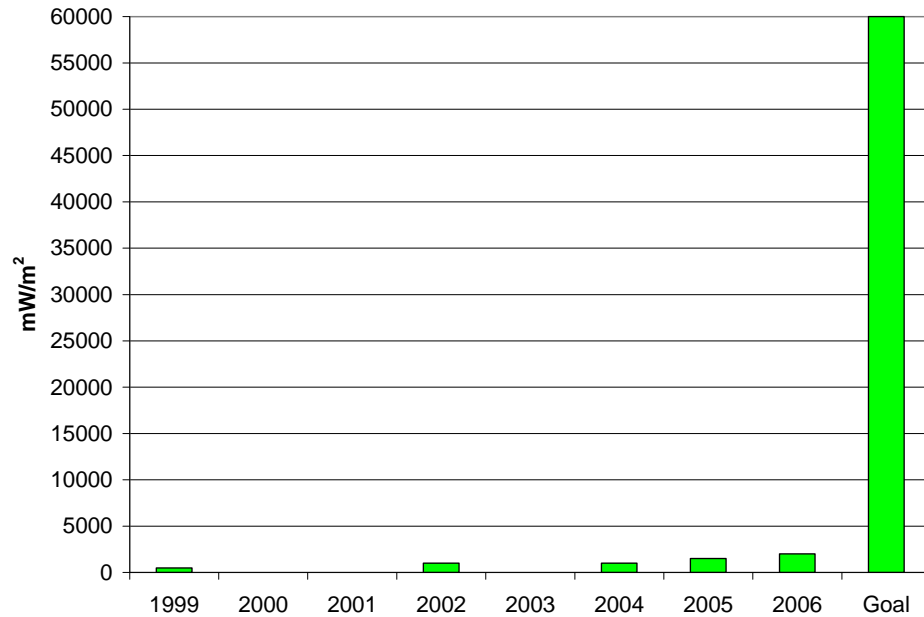
A recent study performed by a research team from Pennsylvania State University determined the ability of swine wastewater to generate electricity in a bacterial battery while treating the high amount organic contamination ²⁰. They demonstrated that a single-chambered aerated cathode MFC produced higher power densities than that of a double-chambered MFC under the same conditions. Furthermore, they found that swine waste, which contains higher amounts of organic matter, surpassed the power generation densities of domestic wastewater by about 80% ²⁰. The current study focuses on another form of waste, raw sewage from domestic areas, and its ability to first generate current using a bacterial battery, then the later possibilities for using the electricity from the bacterial batteries to generate storable hydrogen gas for later use.

Researchers constantly study ways that may improve or optimize the efficacy of bacterial batteries. The highest energy power output reported to date from a bacterial battery fueled by sewage reached 464 milliWatts/m² (mW/m²) of anode surface area ²³. However, in order for these batteries to become realistic, even on a small-scale level, the maximum power output needs to reach at least 1 W/m² ⁽⁷⁾. Figure 1 represents the power output needed in order for bacterial batteries to become

commercially and economically viable. Studies suggest that providing a larger surface area on the anode for microorganisms to live could possibly strengthen and increase the current production^{19, 22, 25}. The current project includes a newly designed bacterial battery that contains electrodes with different surface areas in attempt to document differences in current production. Other engineering challenges related to development of efficient batteries have to do with location of electrodes and the PEM¹⁰. Cheng et al. (2006) was the first research team to produce the highest amount of power in a flow-through system converting liquid waste to energy²³. Their study proved that the energy output reached a maximum 2 W/m² of the anode surface area. However, some uncertainty remains as to whether the system can produce enough power to account for the energy expended for initial pumping²³. The current study tested the efficacy of a batch battery system instead of the previously mentioned flow-through system.

Figure 1: *The bar graph below illustrates the energy output in mW/m² of anode surface from bacterial batteries operating on raw sewage. A goal of 60,000 mW/m² is needed in order for bacterial battery systems to become economically viable.*

(Estimations courtesy of John Lucentini ⁷)



Current research

The current research sought to develop a uniquely designed bacterial battery capable of utilizing sewage for the production of electrical current. Previous bacterial battery studies have primarily focused on the potential energy available in oxidizable organic substances in the form of river sediment, salt marsh and other marine sediments, and animal wastewater. Because of the limited amount of literature available, this study is distinct in its effort to specifically test the efficacy of sewage within a bacterial battery system. This new concept of utilizing wasted energy found

within the overabundant supply of raw sewage, which is generally viewed as simply a pollutant, seems promising upon the continuance of further studies. Another goal was to test the use of these batteries in the electrolytic production of hydrogen gas.

Ultimately, through the optimization of these bacterial batteries, they may one day have an applicable role in household septic tanks as a potential source of electricity.

Earlier research performed by Dr. Henry Spratt, a microbiologist at the University of Tennessee at Chattanooga (UTC), along with a team of research students, sought to establish bacterial batteries using Tennessee River riparian wetland sediments and Chattanooga Creek sediment (a local creek, sections of which have been designated a Superfund site by the U.S. EPA). The bacterial battery system was composed of three graphite anodes and three graphite electrodes with a smaller surface area than the electrodes used in the current study. Current production in these batteries was tested at the expense of the mineralization of various organic compounds, such as polycyclic aromatic hydrocarbons (PAH's), glucose, and various fermentation end products. Their findings suggested that bacterial batteries derived from these sediments with the addition of organic compounds could successfully generate electrical current. Another trial run by Spratt and his students used sewage sludge from the Cleveland, TN City sewage treatment plant as the organic source to fuel bacterial batteries. In fact, success in generating electrical current with this sewage sludge gave Spratt and other students the idea to try raw sewage as the organic fuel for future bacterial batteries. This study represents the next generation of bacterial batteries in Dr. Spratt's lab, using raw sewage to fuel the bacterial batteries.

The current research sought to modify the battery design used in previous UTC studies to create a new and more effective system. Upon completion of the study, results should ideally provide suggestions for more improved battery systems. In addition to structural modifications, the current study represents one of the first applications for the effectiveness of using raw human sewage in generating electrical current. Furthermore, it proposes the concept of using these bacterial batteries to drive the electrolytic production of hydrogen gas that could be stored for later use. These concepts are significant in their contribution toward future bacterial battery research that may one day lead towards the development of an economically and effective source of renewable energy and possibly towards a hydrogen-based economy in the distant future.

Methodology and Materials:

Battery set-up

The newly designed system in this study required the use of six ten-gallon aquaria. The six aquaria were stored on wooden shelves capable of holding their weight at maximum capacity (Fig. 4). Construction of the bacterial batteries began by creating scaffolding used to stabilize and hold the graphite electrodes inside the tanks. The scaffolding was assembled manually using one-half inch PVC piping, T-PVC connectors, PVC pipe adhesive, and PVC pipe cutters. The final scaffolding would thus create an approximate four-inch vertical margin between the cathodes and anodes once in place. Using an electric drill, small notches were drilled into the horizontal shelf of the scaffolding to allow for the two anodes, as well as the two cathodes, to sit on the scaffolding without shifting during the experiment (Fig. 2).

The graphite electrodes, as purchased, were 1.25 inches in diameter and about 1.0 m in length. Since they were too large for the aquaria, they were cut into twenty-six, sixteen-inch electrodes to fit the dimensions of the tank. In order to determine the affects of surface area on current generation, the surface area of the electrodes in three of the batteries was increased by drilling holes into them. A series of 4.0 mm diameter holes spaced 1.0 cm apart were drilled into each electrode. These drilled electrodes were placed into batteries designated I, II, and III and possessed an average surface area of 0.054 m² each. The electrodes in batteries IV, V, and VI were left solid to act as a control and possessed a surface area of 0.045 m² each. Another set of holes, 1.0 mm in length, was drilled into one end of each of the sixteen electrodes.

This was necessary in order to acquire a deep and secure connection between the wire and the electrode. To insure a secure connection, KOPR-SHIELD, a conductive grease, was added to the drilled holes prior to the addition of the wires. After inserting the wires and KOPR-SHIELD, marine epoxy was used to secure and seal the wiring into the holes of each electrode. Next, the wires of the four graphite electrodes from each tank were connected outside the tanks; two wires from the two anodes were secured with wiring nuts, while the two cathodes were also secured with wiring nuts outside the tanks. All four wires in each tank were then connected together to that tank's multimeter device capable of detecting current in microamps (Fig. 3). Only four meters were successfully connected to the computer to allow for recordings to be taken via a data logger. Recording for the remaining two multimeters not connected to a computer were taken periodically throughout the day by hand. A semi-porous Plexiglas barrier was then inserted between the anodes and cathodes to create an anoxic environment for the bacteria on the anodes (Fig. 2). Each 10-gallon aquaria was filled with eight gallons of distilled water to be used to equilibrate the system. Finally, in order to assure proper aerobic conditions for the *Geobacter* on the cathodes, rubber tubing was assembled so each tank could have air bubbled into the battery. The air was released just below the surface of the water. Dissolved oxygen (dO_2) and pH readings were documented throughout the entire study to test whether the oxygen levels near the cathodes remained higher than that of the anodes.

Figure 2: *The bacterial battery design included a Plexiglas barrier, two graphite cathodes overlying the two graphite anodes. This picture further illustrates the wiring connections and bubbling system in the top left corner.*

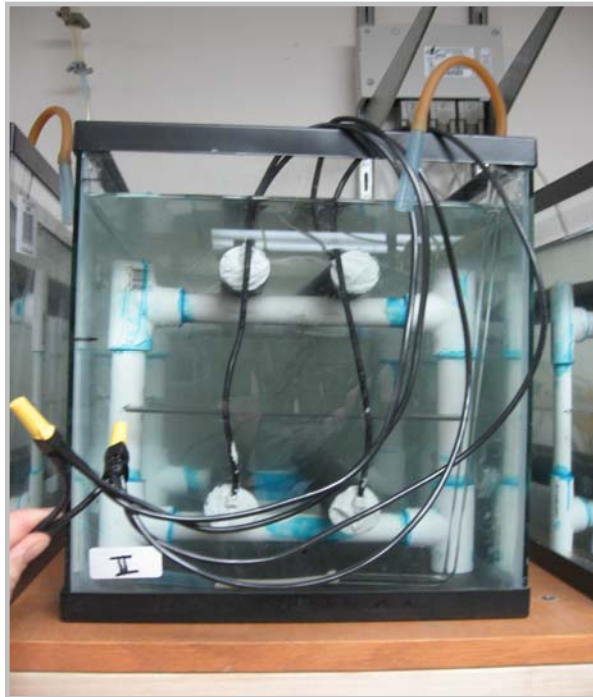


Figure 3: *Multimeter used to record currents in microamps.*



Figure 4: *Final system set-up, including shelving and computer connections.*



Initial battery testing for equilibrium

Initial testing of the bacterial batteries required filling the empty tanks with eight gallons of distilled water in order to test for stability before adding the sewage. Another series of control experiments were then performed in preparation for the addition of sewage. An initial inoculation of sediment was extracted from the central cell of an already operating bacterial battery from a previous study at the University of Tennessee at Chattanooga. Tennessee River riparian wetland sediments were used in this early bacterial battery from Dr. Henry Spratt's lab. A thoroughly distributed slurry containing 5.0 mL of the aforementioned sediment and approximately 195 mL of 0.85 % saline were combined in a 250 mL Erlenmeyer flask and prepared using a magnetic stirrer at medium power. Once evenly disseminated, 5.0 mL of the slurry was inoculated into each bacterial battery. After settling in the water-filled tanks, 1.0 mL of concentrated glacial acetic acid was added directly above the Plexiglas in each tank using a 5.0 mL pipette. Any significant bacterial metabolism occurring could be exhibited via current generation. Current was recorded every 3 minutes over a time span of 30 minutes.

A second addition of acetic acid, approximately 5.0 mL, was carefully inserted into the anoxic chamber of the tanks using a 10.0 mL pipette. Following this inoculation, records of current readings were taken every 5 minutes over a time span of 75 minutes. After effectively testing the effects of adding acetic acid to the tanks, the bubbling was suspended to evaluate whether too much circulation was affecting the anaerobic conditions near the anodes.

Battery testing using sewage

The Moccasin Bend Sewage Treatment Plant in Chattanooga, TN supplied the sewage for each of three experiments. Specifically, the sewage was collected from the duct immediately following the debris removing filters and preceding the primary treatment tanks. The same procedures were applied for the three additions of sewage. Each addition of sewage required the collection of fifteen gallons of sewage distributed evenly within three five-gallon carboys. Each of the three collecting carboys were labeled A, B, and C. Before the direct addition of sewage into the tanks, three 10.0 mL samples from each thoroughly mixed carboy were taken. Using a 10.0 mL pipette, three samples from each carboy (labeled A, B, and C) were injected into individual sample vials labeled carboy A1, A2, A3, B1, B2, B3, C1, C2, and C3. All sample vials were then put into the freezer at $-20\text{ }^{\circ}\text{C}$ in order to be preserved for later use. In time, the samples could then be poured into crucibles and dried for two days in a drying oven at $60\text{ }^{\circ}\text{C}$. Once completely dried, the samples were reweighed and put in a muffle furnace at $500\text{ }^{\circ}\text{C}$ for two hours. The burned samples were then reweighed to accurately calculate the particulate percent organic matter present in the solid matter present in sewage in each specific carboy. This allowed comparisons to be made concerning the initial difference in organic content and the subsequent current generation. By identifying the primary organic content in each and by keeping track of which carboy sewage samplings were added to which batteries, the chemical properties of the water and level of organic matter in each tank could be calculated and monitored. During the second and third experiment, 10.0 mL

water samples were retrieved from the top (cathode chamber) and bottom (anode chamber) of each battery in order to determine the organic matter present in the water throughout the trial. This process was repeated two times for experiment two and three. Additionally, dissolved oxygen (dO_2) and pH levels were observed and recorded at two specific points during each experiment. A YSI 556 Multi Probe System was used to obtain these readings.

For the first experiment, each tank was filled with six gallons of distilled Tennessee River water and two gallons of raw sewage. For the remaining two experiments, the tanks were siphoned to the six-gallon mark and then refilled with two gallons of fresh sewage. In the case of the second and third additions, some of the drained water content may have included small, but primarily, insignificant amounts of organic matter. (Note: sewage from carboy A was deposited into tanks I and II; sewage from carboy B was deposited into tanks III and IV; sewage from carboy C was deposited into tanks V and VI).

The duration of each experiment was variable due to inconsistencies with computer programming and memory and complications with scheduling. With the first addition of sewage, the experiment lasted approximately 14 days. The second experiment ran for 19 days. The final trial lasted 13 days. Current recordings for tanks I, II, IV, and V were automatically recorded into a computer data logger program. The data logger program collected current measurements every minute. The amount of current generated in tanks III and VI was manually recorded by hand every thirty minutes for approximately four hours during the middle of the day.

Unexpected computer malfunctions and loss of records taken because of manual data logging led to some lapses in data acquisition.

Electrolytic cell testing

During initial preparations, an electrolytic cell was set up and tested. Due to the malfunctioning platinum electrodes in this electrolytic cell, graphite electrodes that were 9.0 mm in diameter and 10.0 cm in length were used as their replacement. Synthetic “O”-rings of 9.0 mm diameter were used to secure the graphite electrodes in place inside the burettes of the electrolytic cell. These electrodes were connected through holes drilled outside the burettes to a power source. After pouring distilled water into the apparatus, it was determined that the newly devised apparatus was watertight. The water was emptied and replaced with 0.85% NaCl solution.

In order to see if the electrolytic cell setup would operate, the wiring from each of the two electrodes was connected to a single 1.5 V D-battery to test whether these electrodes would produce hydrogen and oxygen (one wire was connected to the positive side and one wire was connected to the negative side).

In order to further test the applicability of the electrolytic cell in splitting water molecules into oxygen and hydrogen, a power supply device (FisherBiotech Electrophoresis System model FB 150) capable of providing power to the electrolytic cell was connected. Three experiments were performed. For the first experiment, fresh 0.85% saline solution was injected into the cylinders of the cell. The power supply was turned on for four hours at full power. In the second experiment, fresh

0.85% saline solution was again added to the cell and the power supply was turned on for six hours. A third experiment was performed after altering the circuits to ensure that no problems pertaining to the positive and negative connections on the electrodes existed. Again, fresh 0.85% saline solution was added and the power supply remained on for six hours.

Results:

Initial battery testing

Initial current readings in the batteries read below $0.2 \mu\text{A}$ with the addition of distilled water only. Following the inoculation of bacterial slurry, the addition of 1.0 mL acetic acid increased currents slightly. Figure 5 illustrates the immediate current generation in the tanks. After the second injection of 5.0 mL acetic acid, currents from all six tanks increased within the first thirty minutes (Fig. 6). Two days following the additions of the acetic acid, all tanks displayed turbidity and bacterial growth on electrodes.

Figure 5: *Current generation after addition of 1.0 mL glacial acetic acid (indicated by the arrow). Current was recorded every three minutes over a time span of 30 minutes.*

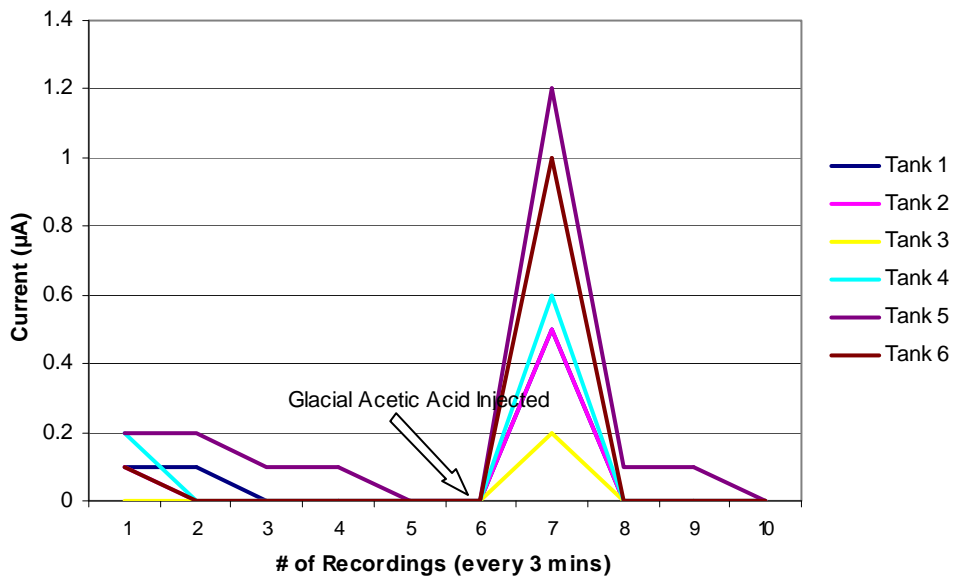
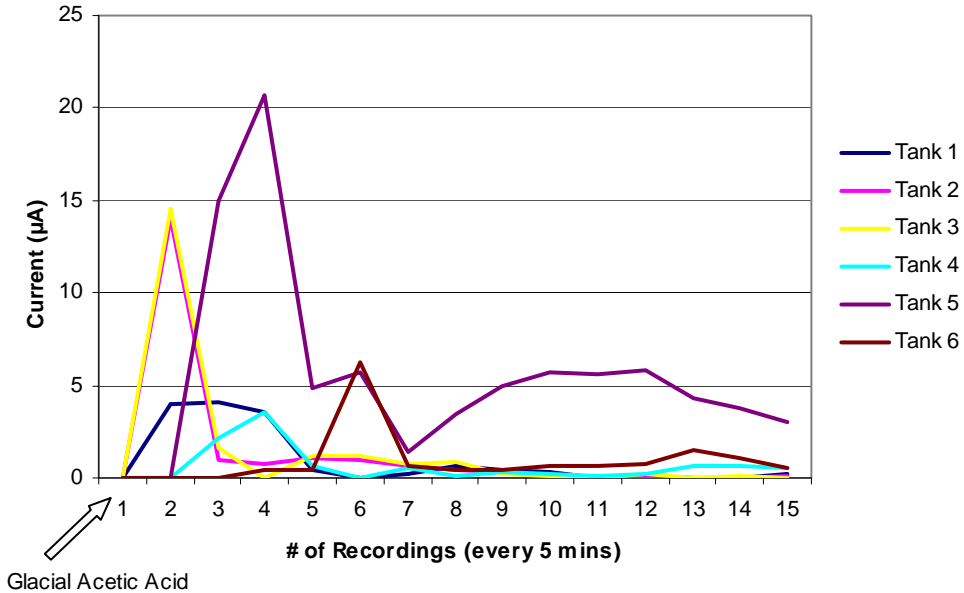


Figure 6: *Current generation after addition of 5.0 mL glacial acetic acid (indicated by the arrow). Current was recorded every five minutes over a time span of 75 minutes.*



Battery testing using sewage

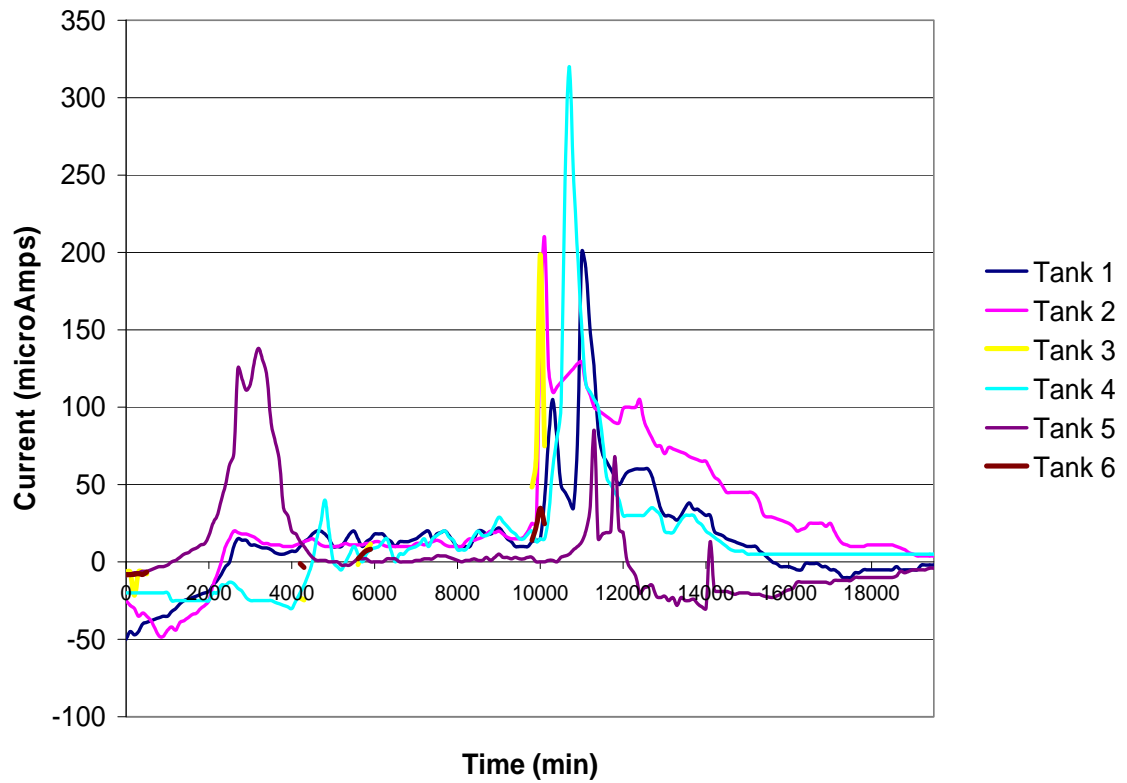
Results for each of the main three experiments include current generation after the addition of raw sewage, dissolved oxygen (dO₂) levels (ppm), pH readings, percent particulate organic matter in the initial supply of raw sewage, and percent organic matter in water samples retrieved from operating bacterial batteries at certain times throughout the experimental process.

Experiment I- First addition of sewage

Current generation

Figure 7 clearly represents the currents in microamps (μA) for each of the six bacterial batteries. Tank V first peaks at 138 μA , while the remaining batteries hover near zero μA . Approximately eight days (~10,700 minutes) into the experiment, a noteworthy peak in currents occurred for all six batteries. Tank IV generated the most current of 320 μA , while tanks I, II, and III reached a maximum of about 200 μA . Tanks V and VI remained below 100 μA . After reaching day 14 (19,500 minutes) of the experiment, batteries no longer produced any significant current.

Figure 7: This graph represents current generation recorded in μA after the first addition of the raw sewage. Sewage was added at time zero. Tanks 1 through 3 contain drilled electrodes. Tanks 4 through 6 contain solid electrodes.



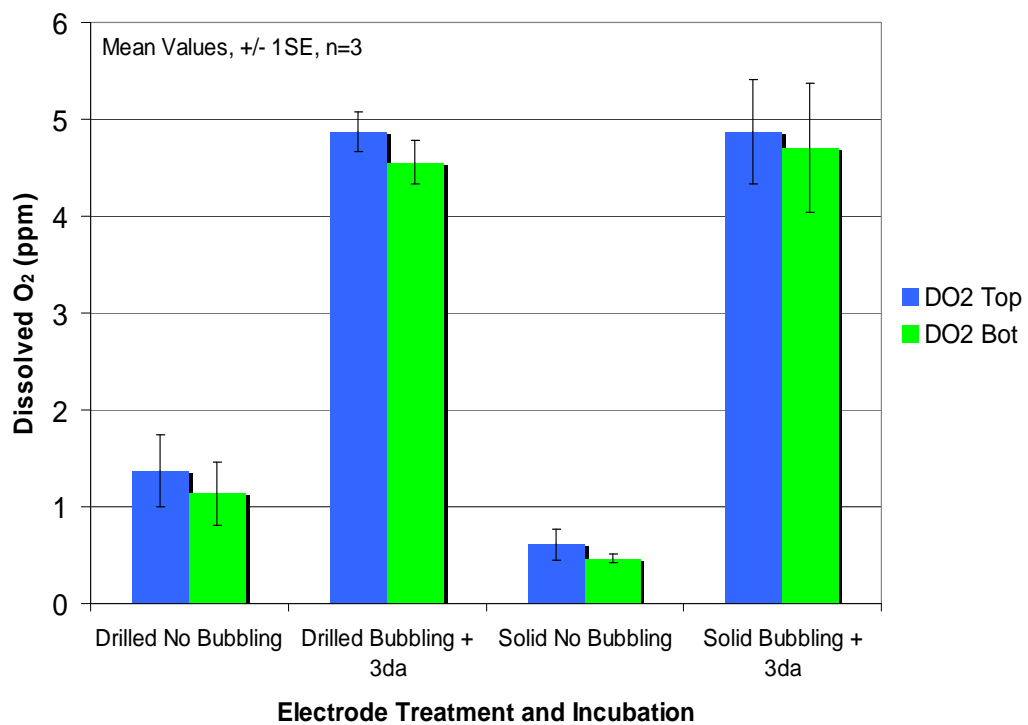
Dissolved Oxygen

Dissolved O₂ and pH levels were observed and recorded for the top and bottom of each battery seven days (10,080 minutes) into the first experiment, while no oxygen was being added to the cathodic chamber. Three days later, after the bubbling system was turned on (i.e. ten days or 14,000 minutes into the experiment), dO₂ and pH levels were again recorded. Figure 8 indicates the average dO₂ level in the top and the dO₂ average in the bottom of the batteries before and after the deliberate addition of oxygen to the system.

Before the addition of oxygen, batteries I, II, and III (containing drilled electrodes) possessed dO₂ levels averaging from 1.4 ± 0.4 ppm in the top to 1.1 ± 0.3 ppm in the bottom. The batteries containing solid electrodes, however, yielded dissolved oxygen levels below 1.0 ppm, indicating anoxic conditions throughout.

The addition of oxygen to the batteries increased the dO₂ levels of both the drilled and non-drilled batteries. The amount of dO₂ in all tanks reached approximately 4.87 ppm in the top and 4.6 ppm near the bottom. Thus, the difference in surface area did not affect the amount of dO₂ present in the tanks.

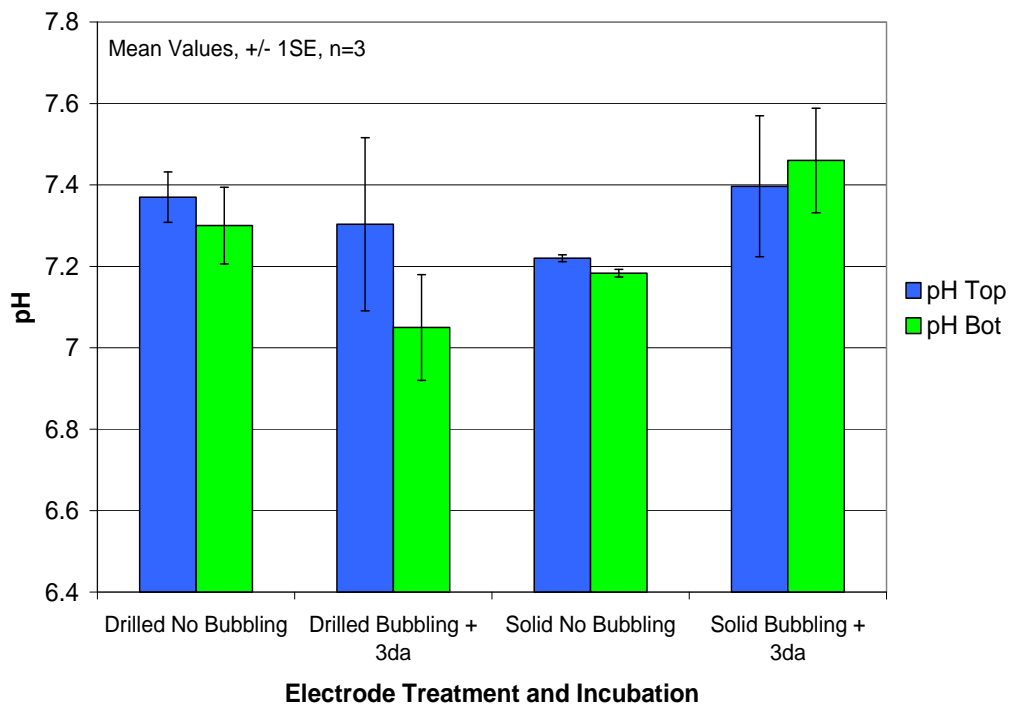
Figure 8: The bar graph represents the dO_2 levels present within the top and bottom of each battery containing either drilled or solid electrodes. Measurements were recorded seven days (10,080 min) into the experiment while no bubbling occurred and ten days (14,000 min) into the experiment after the bubbling system was activated.



pH

Figure 9 illustrates the average pH levels before and after the addition of oxygen. The pH readings from the top of tanks I, II, and III remained at 7.3 before and after the bubbling system was activated. The bottom of these tanks slightly dropped from a pH of 7.3 to 7.1. Prior to receiving oxygen, the pH of batteries IV, V, and VI measured exactly 7.2 in both the top and the bottom. Activating the bubbling system raised the pH to 7.4 in the top and 7.46 in the bottom. For Tanks I, II, and III, the addition of oxygen caused a decrease in pH, where as the presence of oxygen raised the pH for tanks IV, V, and VI.

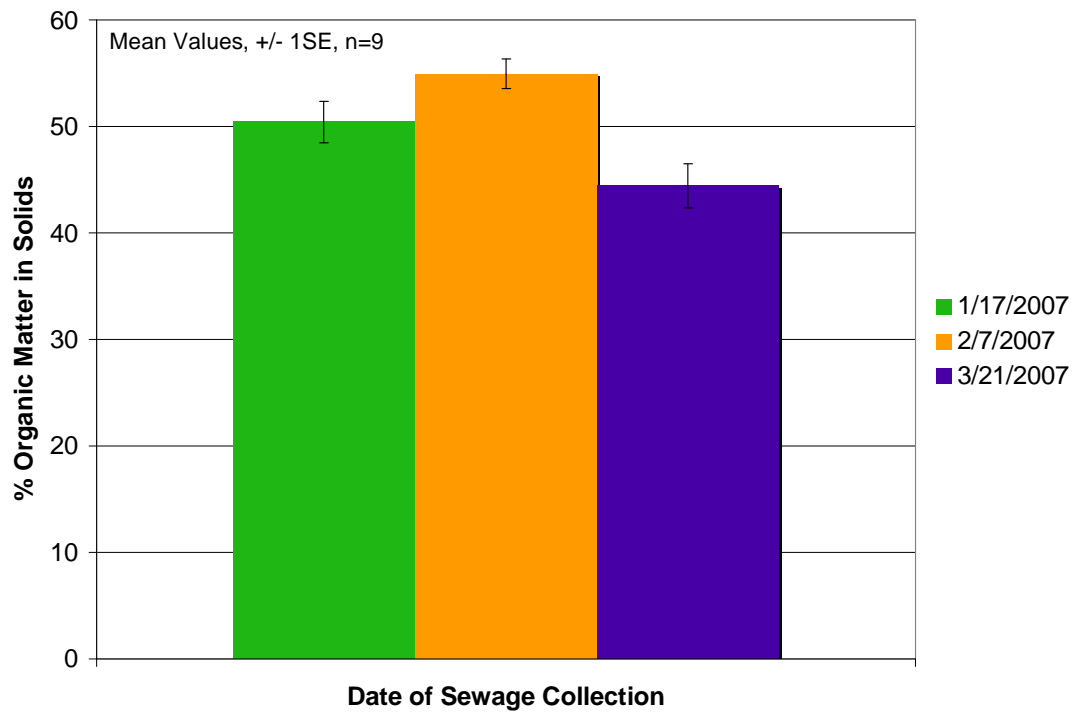
Figure 9: The bar graph below represents the pH levels present within the top and bottom of each battery containing either drilled or solid electrodes. Measurements were recorded seven days (10,080 min) into the experiment while no bubbling occurred and ten days (14,000 min) into the experiment after the bubbling system was activated.



Percent Organic Matter

The particulate material present in raw sewage obtained on 1/17/2007 and used for the first experiment contained 58 ± 1 percent organic matter (Fig.10). No other percent organic matter tests were conducted during the first experiment.

Figure 10: *The bar graph below represents the particulate material present in the initial batches for each of the three experiments.*

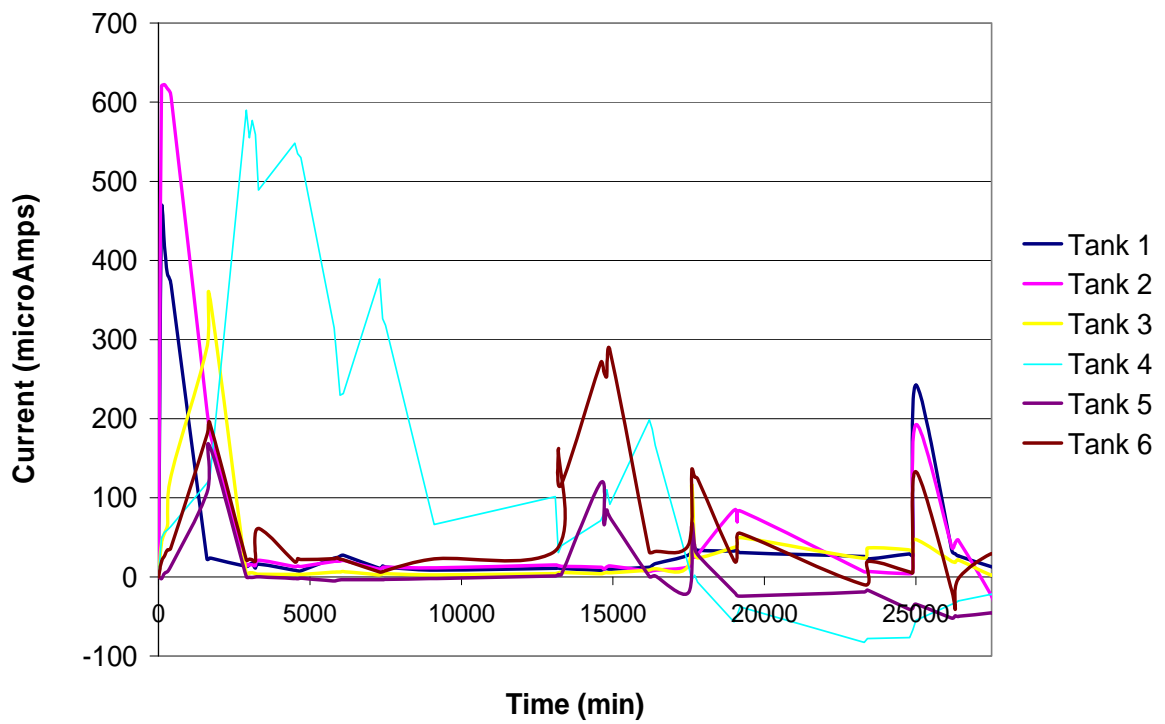


Experiment II- Second addition of sewage

Current generation

Figure 11 depicts the currents in microamps (μA) for each of the six bacterial batteries. The bacterial battery current generation for the second experiment immediately increased in all tanks within the first two days (2,900 minutes). Five hours (300 minutes) into the experiment, tank II produced a maximum current of 622 μA . At 28 hours (1,700 minutes), tank III peaked at 353 μA , tank VI peaked at 194.5 μA , and tank V peaked at 164.7 μA . At two days (2,900 minutes), Tank IV generated a 425 μA current. The currents for all tanks, except for tank IV, subsided to below 25 μA around the fourth day (5,000 minutes). Tank IV remained within the hundred μA range until day six of the trial. Near the eleventh day (15,000 minutes) tanks VI, V, and VI generated currents ranging from 100-300 μA , while the first three tanks produced currents remaining below 100 μA . After the twelfth day (17,000 minutes), tanks VI and V produced negative currents. The last noticeable spike in current generated during the second experiment occurred in tanks I, II, and VI on day 17 (25,000 minutes).

Figure 11: *This graph represents current generation recorded in μA after the second addition of the raw sewage. Sewage was added at time zero. Tanks 1 through 3 contain drilled electrodes. Tanks 4 through 6 contain solid electrodes. Note the negative current generation.*

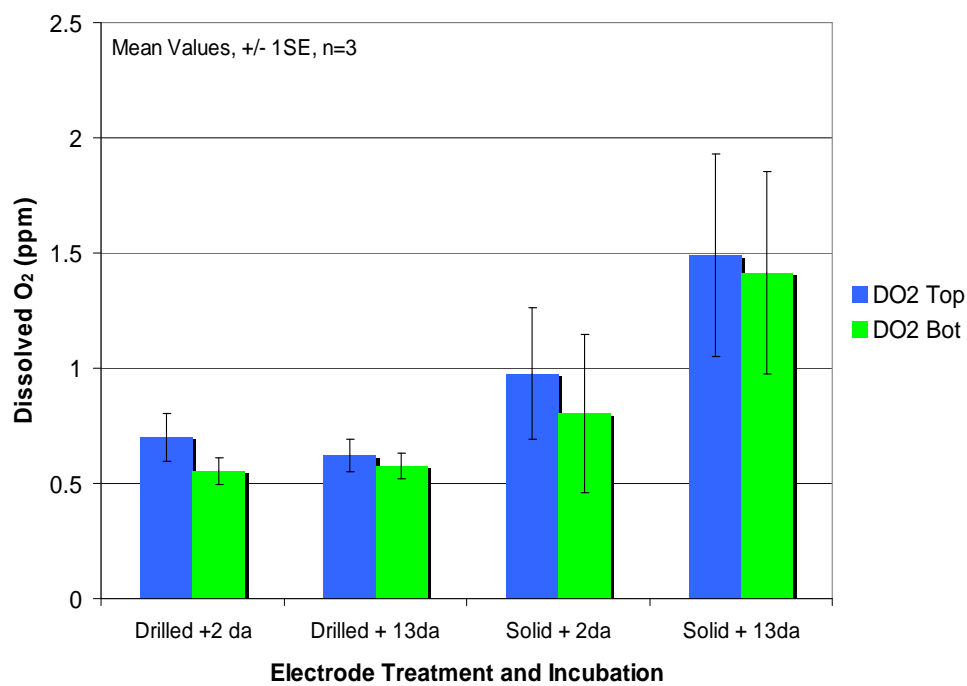


Dissolved Oxygen

Dissolved O₂ was observed and recorded in the top and bottom of each battery two days (2,880minutes) and thirteen days (18,720 minutes) into the second experiment. Figure 12 indicates the average dO₂ level in the top and the dO₂ average in the bottom for both days. Dissolved O₂ was much lower in the second experiment as opposed to the first.

The dO₂ readings recorded on day three fell below 1 ppm in all tanks, indicating that the all batteries possessed anoxic conditions. However, on day 13, average dO₂ measurements for tanks IV, V, and VI (solid) reached 1.5 ± 0.4 ppm in the top and 1.4 ± 0.4 ppm in the bottom, while tanks I, II, and III (drilled) remained less than 1.0 ppm. Overall, the first three batteries possessed dO₂ averages below those of tanks IV, V, and VI.

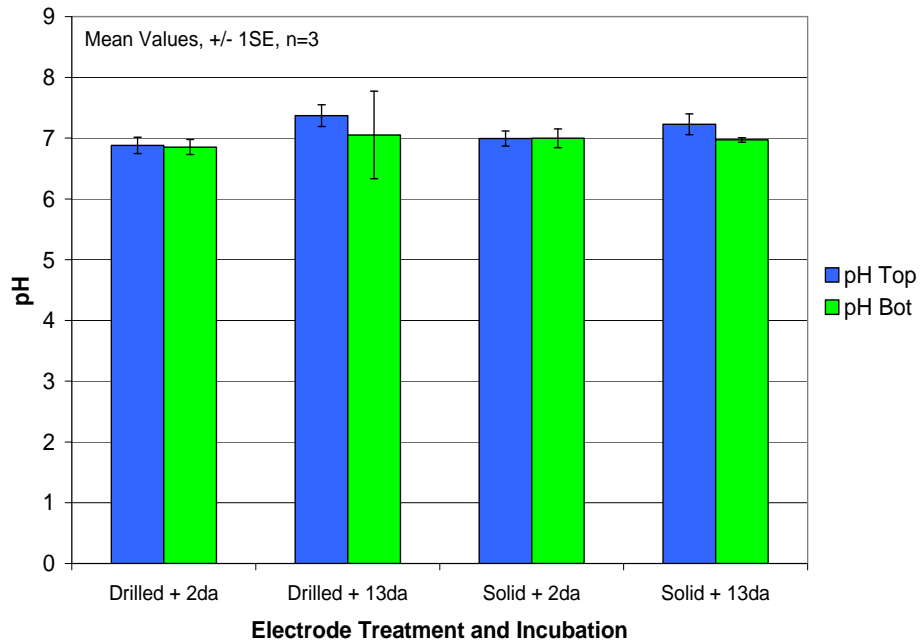
Figure 12: The bar graph represents the dO_2 levels present within the top and bottom of each battery containing either drilled or solid electrodes. Measurements were recorded two days (2,880 min) and thirteen days (18,720 min) into the second experiment.



pH

Figure 13 illustrates the average pH levels taken on day two and day thirteen of the second experiment. Results insignificantly changed during the experiment. The pH in both the top and bottom of all batteries, regardless of change in electrode surface area, remained near the neutral pH of 7.

Figure 13: *The bar graph below represents the pH levels present within the top and bottom of each battery containing either drilled or solid electrodes. Measurements were recorded two days (2,880 min) and thirteen days (18,720 min) into the second experiment.*



Percent Organic Matter

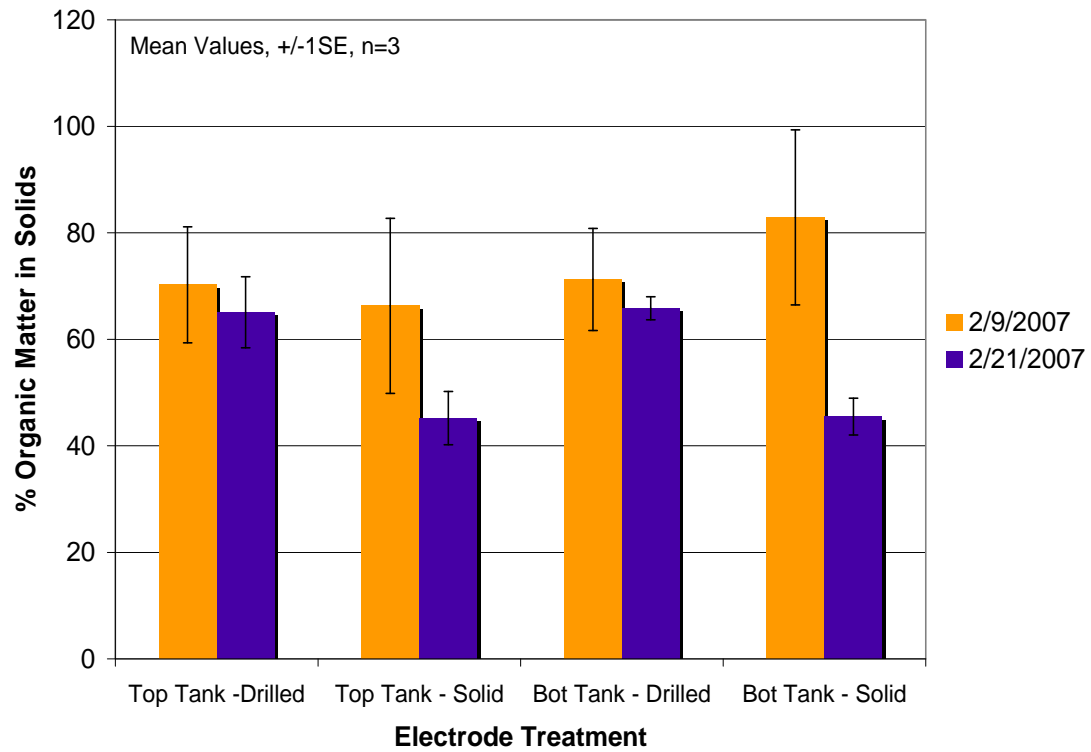
Particulates present in the raw sewage obtained on 2/7/2007 and used for the second experiment contained 48 ± 3 percent organic matter (Fig. 10) prior to its introduction into the bacterial batteries.

Figure 14 represents the percent particulate organic matter found in water samples taken from the top and bottom of every tank on two different dates. Comparisons in organic matter can be made between tanks I, II, and III with drilled electrodes and tanks IV, V, and VI with solid electrodes.

For tanks I, II, and III (drilled), the average percent organic matter present in liquid from the top and bottom of each tank was approximately the same on both retrieval dates. On 2/9, the % organic matter for the top equaled 70 ± 11 , and the bottom equaled 71 ± 10 . On 2/21, the % organic matter for the top equaled 65 ± 11 and the bottom equaled 66 ± 2 .

For tanks IV, V, and VI (solid), percent organic matter on 2/9 in the top tank was 66 ± 10 , while the bottom of the tanks displayed a higher average approximating 83 ± 16 . Water samples retrieved from the top and bottom portions of the tanks (solid) on 2/21 contained analogous amounts of organic matter. The top contained 45 ± 5 , where as the bottom contained 45 ± 3 .

Figure 14: *The bar graph below represents the percent particulate organic matter found in water samples taken from the top and bottom of every tank on two different dates during the second experiment.*



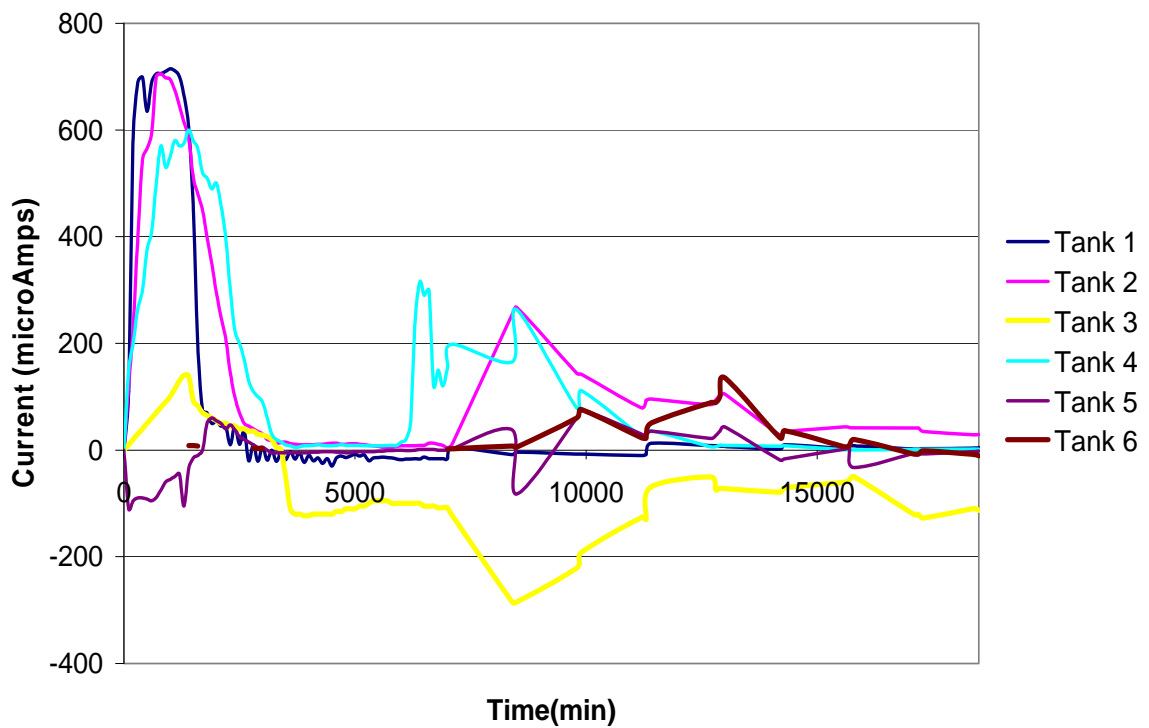
Experiment III- Third addition of sewage

Current Generation

Figure 15 depicts the currents in microamps (μA) for each of the six bacterial batteries after the third addition of raw sewage. The current generations for the third experiment immediately increased in tanks I, II, III, and IV within the first day. Approximately 6.6 hours (400 minutes) into the experiment, tank I produced a current of 699 μA that increased to a maximum current of 711 μA at 18 hours (1100 minutes). Tank II produced a current of 705 μA at 13 hours (800 minutes). Tank IV generated an initial current of 570 μA at 13 hours (800 minutes), but increased to 600 μA by 24 hours (~1400 minutes). At 24 hours, tank III generated a lesser current of 138.7 μA then unusually dropped below zero. Tanks V and VI registered currents near zero. Tank III generated negative current throughout the majority of the experiment.

Tanks II and IV were the only two batteries exceeding 200 μA past initial current generations. Between day four and day six, tank IV produced a current of 315 μA , and tank II displayed a current of 263 μA . The last noticeable current generation of 135.6 μA occurred in tank VI near day nine (13,000 minutes). Aside from the noted peaks in current generation, all other currents remained under 100 μA and slowly declined throughout the remainder of the experiment

Figure 15: *This graph represents current generation recorded in μA after the third addition of the raw sewage. Sewage was added at time zero. Tanks 1 through 3 contain drilled electrodes. Tanks 4 through 6 contain solid electrodes. Note Negative current generation.*



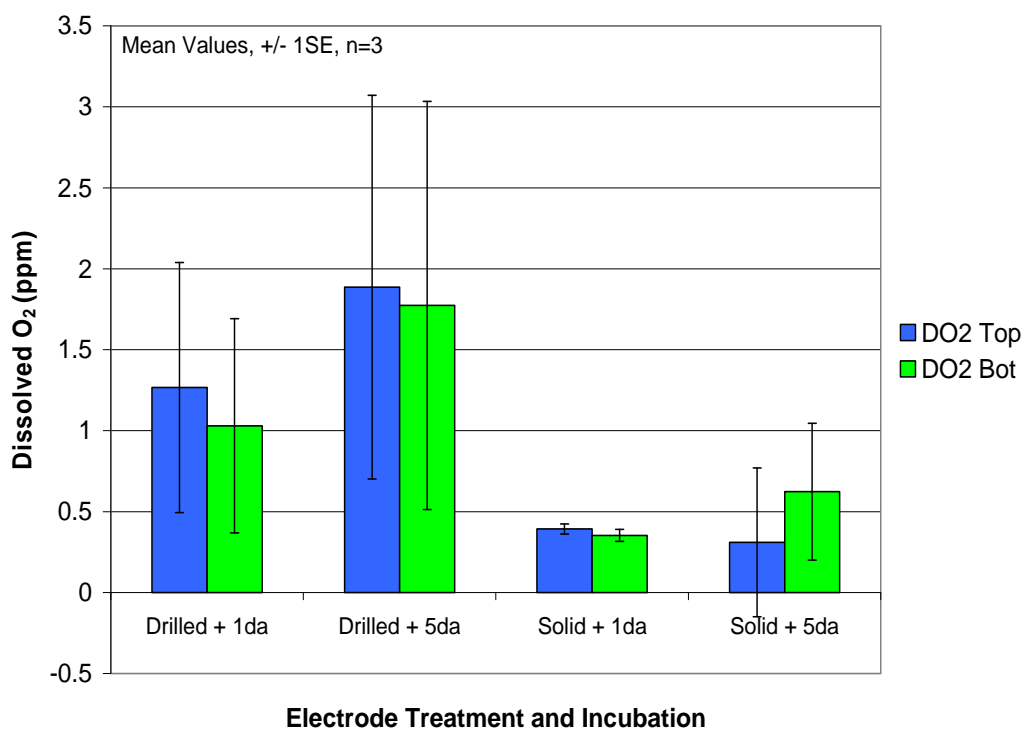
Dissolved Oxygen

Dissolved O₂ (ppm) and pH levels were observed and recorded in the top and bottom of each battery one day (1,400 minutes) and 5 days (7,200 minutes) into the third experiment. Figure 16 indicates the average dO₂ level in the top and the dO₂ average in the bottom for both days.

On day one, the dO₂ measurements for tanks I, II, and III (drilled) averaged higher than 1 ppm, where as tanks IV, V, and VI (solid) fell below 0.5 ppm. For the first three tanks, the dO₂ equaled 1.3 ± 0.8 and the bottom equaled 1.0 ± 0.7 .

On day five, the dO₂ measurements for tanks I, II, and III (drilled) again reached levels higher than 1 ppm, where as tanks IV, V, and VI (solid) fell below 1 ppm. Dissolved O₂ averages for tanks I, II and III increased since the first day with averages in the top reaching 1.9 ± 1.0 and averages in the bottom reaching 1.8 ± 1.3 .

Figure 16: The bar graph represents the dO_2 levels present within the top and bottom of each battery containing either drilled or solid electrodes. Measurements were recorded one day (1,400 minutes) and 5 days (7,200 minutes) into the third experiment.

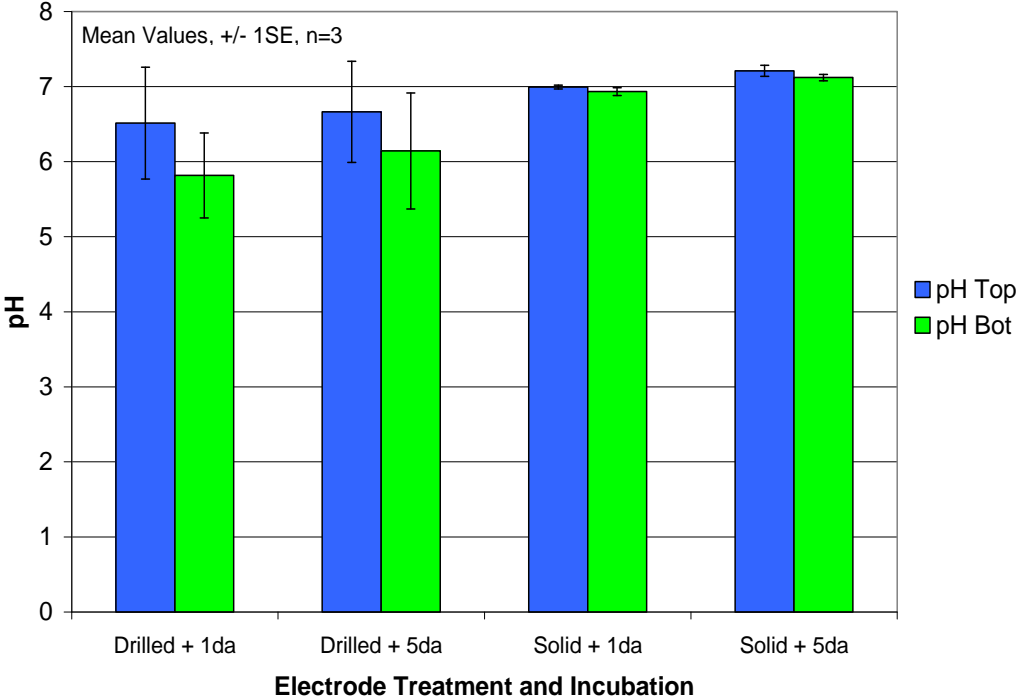


pH

Figure 17 illustrates the average pH levels taken on day one and day five of the third experiment. On day one, the pH averages fell below 7 for tanks I, II, and III (drilled) and remained neutral (~ 7) for tanks IV, V, and VI (solid). For the drilled batteries, the pH was 6.5 ± 0.7 in the top and 5.8 ± 0.6 in the bottom.

On day five, the pH averages were below 7 for tanks I, II, and III (drilled) and again remained near the neutral pH of 7 for tanks IV, V, and VI (solid). For the drilled batteries, the pH was 6.7 ± 0.7 in the top and 6.1 ± 0.8 in the bottom.

Figure 17: The bar graph below represents the pH levels present within the top and bottom of each battery containing either drilled or solid electrodes. Measurements were recorded one day (1,400 minutes) and 5 days (7,200 minutes) into the third experiment.



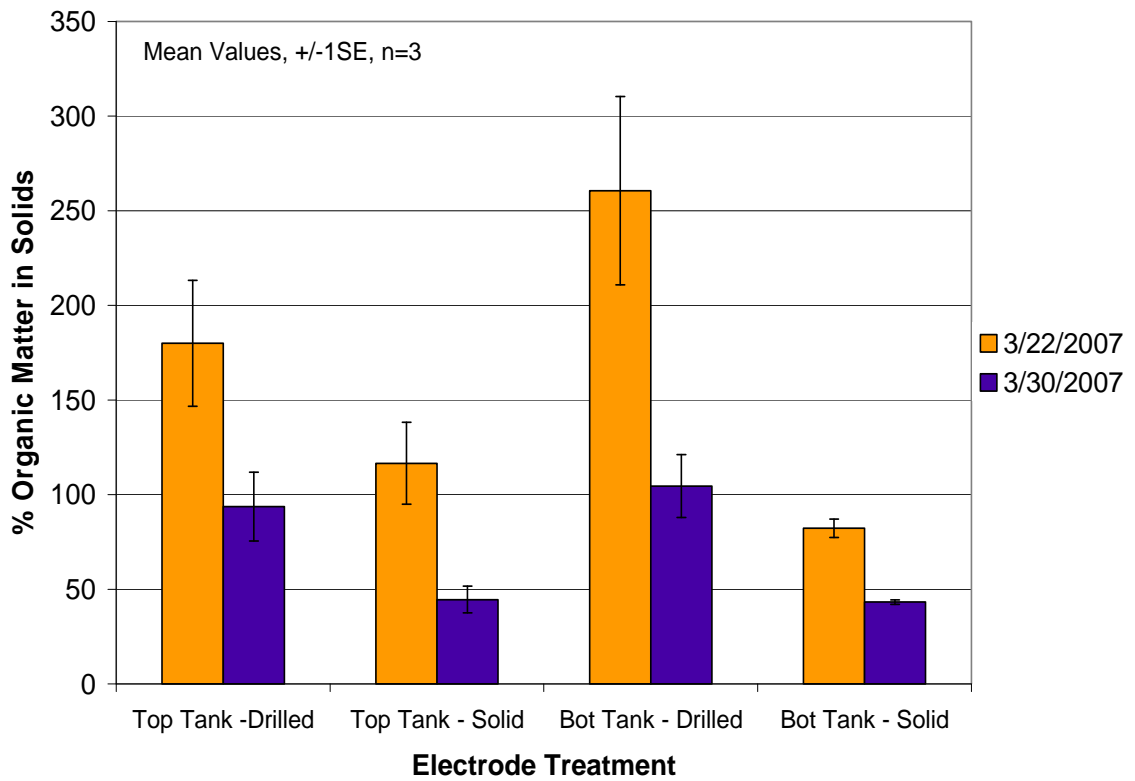
Percent Organic Matter

Particulates present in the raw sewage effluent used in the third experiment and collected on 3/21/2007 contained 46 ± 1 percent solid organic matter (Fig. 10) prior to its introduction into the bacterial batteries.

Figure 18 represents the percent organic matter found in water samples taken from the top and bottom of every tank on two different dates. Comparisons in organic matter can be made between tanks I, II, and III with drilled electrodes and tanks IV, V, and VI with solid electrodes. On 3/22, the amount of organic matter in the top of tanks I, II, and III (drilled) was significantly lower than the amount present in the bottom. The top of the tanks averaged 180 ± 30 and the bottom averaged 261 ± 50 percent organic matter. The amount of organic matter in the top of tanks IV, V, and VI (solid) was estimated higher than the organic material in the bottom. The top of the tanks averaged 117 ± 22 and the bottom averaged 82 ± 5 percent organic matter. Overall, tanks I, II, and III contained higher percentages of particulate organic matter

On 3/30, the organic matter in all tanks was significantly less than the averages recorded on 3/22. The amount of organic matter in the top of tanks I, II, and III (drilled) was only slightly lower than the amount present in the bottom. The top of the tanks averaged 94 ± 18 and the bottom averaged 105 ± 50 percent organic matter. The amount of organic matter in the top of tanks IV, V, and VI (solid) were approximately equivalent. The top of the tanks averaged 45 ± 7 and the bottom averaged 43 ± 1 percent organic matter.

Figure 18: *The bar graph below represents the percent particulate organic matter found in water samples taken from the top and bottom of every tank on two different dates during the third experiment.*



Hydrogen generation

During initial testing for a period of five days, the D-battery failed to produce hydrogen or oxygen via the electrolytic cell. The potential needed for the electrolysis of water must exceed -2.06 V. Since a D-battery contains a potential of only 1.5 V, it did not provide enough power to produce any gases.

Three separate experiments were performed using the electrolytic cell and a power source capable of producing adequate current and voltage to support electrolysis. In the first experiment, after running the power supply for four hours, the production of hydrogen at the cathode was 4.1 mL, and the amount of oxygen produced at the anode was only 1.9 mL. These results are roughly consistent with the reaction stoichiometry of H₂O. The second and third experiments produced zero gases after being connected to the power supply for six hours.

Table 1: *The table below represents the amount of hydrogen produced during basic electrolytic testing.*

Time	0 min	4 hrs	6 hrs
<i>H₂ produced during Experiment I</i>	0 mL	4 mL	--
<i>H₂ produced during Experiment II</i>	0 mL	0 mL	0 mL
<i>H₂ produced during Experiment III</i>	0 mL	0 mL	0 mL

Discussion:

It was hypothesized that *Geobacter* present in untreated sewage will metabolize organic matter, and given an appropriate graphite electrode, will produce an electric current that could further be used to produce hydrogen gas via electrolysis. Data generated in this study supports this hypothesis to the extent that current production did occur within the bacterial batteries using raw sewage. However, whether or not the electrical current produced in these cells could sufficiently produce hydrogen gas via electrolysis of water is unclear, due to complications involving operation of the electrolytic cell. Ultimately, hydrogen could be efficiently produced and stored for later use by using the current generated in a bacterial battery to power an electrolytic cell. This study succeeded in providing a basis and suggestions for future laboratory studies aimed at creating an improved sewage-powered bacterial battery.

One of the major modifications to the current system was an increase in anode surface area. In previous research performed by Dr. Henry Spratt, a microbiologist at the University of Tennessee at Chattanooga (UTC), anode surface area was 0.0425 m². The current study slightly increased the surface area of the solid electrodes to 0.045 m² and the drilled electrodes to 0.054 m². Studies suggest that providing a larger surface area on the anode for microorganisms to live could possibly strengthen and increase the current production^{6, 17, 19}. An increase in electrode surface area allows for more substantial growth of biofilms, or aggregation of microbes, on these electrodes. In a study performed by Reguera et al. (2006), current generation

increased when the population of *Geobacter* was maximized and packed into a certain volume²⁵. During each of the current experiments involving raw sewage additions, average current generated was higher in the bacterial battery cells that possessed higher surface areas (drilled electrodes) than in cells containing solid electrodes. Particulate organic matter present in the batteries containing solid electrodes declined over time, indicating reduced biofilm growth and lower current production. These findings support earlier research performed by Henry Spratt who has found in other bacterial battery cells that surface area is directly proportional to current generation. Further research by Chaudhuri and Lovely (2003) suggest that the utilization of foam electrodes, in place of graphite electrodes, allow for the attachment of higher concentrations of microorganisms¹⁹.

Comparisons can be made between particulate organic matter in liquid removed from the battery cells in relation to the current generated by the cells. During the first experiment in which raw sewage was added, current production was less immediate and smaller than the current generated in the second and third experiments. Although the initial percent particulate organic matter was lower in the third batch of sewage, currents were still higher. These results suggested that bacterial biomass on the electrodes increased as aliquots of sewage were introduced into the cells. Kim et al. (2005) suggest that the inoculation of bacterial colonies from anodes in a preexisting bacterial cell onto anodes in a new cell could increase energy generation to 40 mW/m² (or 70 mV), which would otherwise only produce 8 mW/m² using unacclimated sewage²². Their results suggested that without the

frequent addition of sewage, electricity production using a bacterial battery will decrease as the organic matter in the wastewater is consumed. Furthermore, they suggest that increased current generation resulted from fresh additions of sediment containing an organic compound such as acetate ²².

The present research demonstrated that current generation increased and was more immediate upon addition of sewage as the bacterial biofilms aged within the cells. This suggests that better battery performance could be obtained using a battery system having a constant flow of raw sewage, such as a septic tank. The development of a flow-through, or chemostat, system capable of producing enough current will require scaling laboratory studies to life size models. In this study, the battery cells used held a maximum of ten gallons of liquid each and followed the designed of a batch system. Only three additions of raw sewage were added to the system over a period of approximately two months. The current study further suggests that human sewage can be used as the organic fuel in bacterial batteries. The average sized septic tank can hold up to 1,000 gallons of waste and is constantly being replaced with new additions of fresh sewage. Sewage treatment additionally requires the use of a flow-through system. In order for bacterial batteries to become a realistic alternative as a renewable energy source, future studies need to focus on scaling up the size of these bacterial batteries to ultimately test their efficacy in life-size residential, industrial, and agricultural settings.

During this study organic matter present in particulates found in the raw sewage and in battery cell water was all that was measured. However, the role of the

particulate organic matter in this system was not easily determined. The study lacked information on the amount of dissolved organic matter or dissolved carbon present in the sewage or battery cell water. *Geobacter* will often metabolize these dissolved compounds, or fermentative waste products, such as acetate, lactate, and some alcohols, while not touching the particulate organic matter¹¹. It is difficult to discuss the conversion of organic matter into electrical current without knowing the total amount of carbon available to these bacteria present in the raw sewage and battery cells. Figure 11 and figure 15 display current peaks toward the middle to the end of the experiments that may be attributed to the further fermentation of particulate organic matter, producing dissolved carbon compounds. Future studies could measure both these types of organic matter to properly assess which type of metabolism is occurring within the battery.

The pH level affecting bacterial growth and metabolism is another important aspect of bacterial batteries. The relationship between pH and current generation is proportional to the relationship between *Geobacter* and fermentative bacteria naturally residing in sewage sediment. The bacterial batteries studied here displayed neutral pH conditions in the majority of the tanks during all three experiments. However, in the third experiment when the highest current levels were generated, pH levels dropped below six in the top drilled tanks (tanks I, II, and III). Acidic fermentation end products generally result in pH values below 6.0. As previously noted, *Geobacter* prefer these fermentation waste products as their food (electron) sources¹¹. As fermentors release increased amounts of organic acids at these low pH

levels, *Geobacter* growth should be stimulated. Thus, these fermentative processes and resulting waste products may be the causal agent of the initial current peaks during the initial acetic acid tests (Fig. 5 and Fig. 6), at the beginning of experiment III (Fig. 15), and towards the end of the second and third experiments (Fig. 11 and Fig. 15). A limited amount of literature exists on the effects of pH in bacterial batteries, so future studies should focus on the implications of pH levels in bacterial growth and its impact on current generation.

Several modifications to the current microbial system could yield improved results. The dissolved oxygen (dO_2) levels within the batteries are another important aspect of study. Throughout this study, dO_2 levels were inconsistent in the battery cells. In a properly working bacterial battery, dO_2 levels should be high in the cathode region of the cell and completely absent in the anode region. However, the batteries used in this study had dO_2 levels that would be inadequate for an efficient bacterial battery (the cathodes were frequently anoxic). It is likely that the high organic content of water surrounding the cathodes diminished the use of these electrodes as electron acceptors. Dissolved O_2 measurements below one ppm are considered anoxic, thus a system needs to be developed in which only the anode region remains anaerobic. Experiment I resulted in an increase in dO_2 levels in the bottom of the tanks as soon as the bubbling system was turned on. In experiments II and III, dO_2 measurements were at times close to 1.0 ppm in both cell chambers, while at other times, possessed aerobic dO_2 levels in both chambers. These inconsistencies could be prevented with an improved battery design with more

complete compartmentalization between the cathode and anode regions.

Furthermore, the fact that several tanks showed reversed current flow (i.e., from the cathode to the anode), suggests that the compartmentalization necessary to encourage *Geobacter* growth on the anodes may not have been produced in this system (Fig. 11 & Fig. 15). Future systems could position cathodes in a tank separate from tanks containing anodes, but connected via a conductive bridge. This should allow the establishment of permanent anaerobic conditions at the anodes and permanent aerobic conditions at the cathodes. Proposed future research at UTC seeks to develop this newly modified battery system and tests its efficacy in comparison with the system used in the current study.

Due to an oversight, the resistance and voltage were not measured while the system was running. This posed a problem in calculating the total power in watts from the current study. Similar voltage and resistance documented in previous research performed by Henry Spratt using wetland sediment was used to estimate total power production for bacterial batteries using riparian sediments. While producing 2.0 mA of current across a voltage of 197.8 mV, a battery using wetland sediment as organic fuel produced a power density of 3.1 W/m² per anode surface area. The maximum current produced by a battery in the current study reached 711 μA (0.705 mA). By extrapolating information provided by the previous study, it was possible to estimate a maximum power output of 2.6 W/m² per anode surface area from the tanks containing higher surface areas (drilled electrodes).

Studies suggest that the maximum current produced by any bacterial battery to date has been 0.1 A²⁸. The average bacterial battery contains a power density of 40 W/m³, with recent optimization reaching 250 W/m³. Past research indicates that typical anaerobic digestion of organic matter can produce approximately 1.0 kiloWatt-hour (kWh) of practical energy, with a power density of 400 W/m³(28). A kWh is 1000 Watts of electricity used for one hour. For example, one kWh hour is needed to light a 100-watt light bulb for 10 hours. Since 110 V x 1 A = 110 W, one amp of current along with 110 volts of AC line potential is needed to power a 100-watt light bulb. The majority of bacterial battery systems do not possess power densities strong enough to even produce one kWh. The current system did not even exceed 1.0 mA of electrical current, which is 1000 times less than the amount of current needed to power an everyday light bulb. Currently, fossil fuels or nuclear fission plants far surpass any capability of bacterial batteries in producing enough power to fulfill routine energy demands. However, as long as fossil fuel powered plants are in operation, detrimental amounts of CO₂ emissions will continue to pollute and harm the atmosphere. Furthermore, limited sources of ²³⁵U used to power conventional nuclear power plants will someday be exhausted. With proper systematic improvements, bacterial batteries have the capability to someday become a reality without exhausting finite resources and posing environmental problems.

Though successful in introducing the concept, the current study encountered several problems with regard to the electrolysis system that was tested as a means to store energy generated by bacterial activity. As results indicated, hydrogen

production only occurred during the first electrolysis testing. In addition to lack of knowledge and inexperience using an electrolytic cell, other technical problems occurred. As previously mentioned, studies suggest that potassium hydroxide (KOH), a highly conductive aqueous solution, serves as the best alkaline electrolyzer in an electrolytic cell. The current study, however, used a 0.85% saline solution instead of KOH, which could have affected hydrogen production. The electrolytic system used here also lacked a membrane separating the electrodes and any gases produced.

Future electrolytic studies should focus on the proper use of these hydrogen generators. Once working properly, these bacterial batteries could directly power these electrolytic cells. Laboratory studies using large-scale bacterial batteries connected to electrolytic cells may produce better approximations on the amount of electrical current needed to produce specific amounts of hydrogen gas. Though perfecting the electrolytic cell is pertinent in creating usable energy, further research needs to provide more knowledge on ways that hydrogen gas can be stored and saved for later use.

The main goals of the current research were reached and may provide contributions to future studies at UTC and elsewhere. A uniquely designed bacterial battery capable of utilizing sewage to produce electrical current was created, and now provides a basis for further improved designs. Additionally, the research introduced the concept of involving bacterial batteries in the electrolytic production of hydrogen gas. Past and present research suggests possible short and long-term applications for

the use of these bacterial batteries as a source of renewable energy. Lovley has proposed the use of these batteries to power small handheld devices, (e.g. calculator)¹¹. Future research will need to examine and perfect methods for acclimating current generation over short time spans in small-scale systems before shifting to large-scale applications. The current study significantly contributed knowledge that may be useful for future bacterial battery research. The amount of knowledge still missing and room for much improvement in the area of electricity production via bacterial batteries allows hope for future studies in developing an efficient and economically viable source of renewable energy.

References:

1. Kursunoglo, B.N., Mintz, S.L., & Perlmutter, A. Global Energy Demand in Transition: The New Role of Electricity. New York, NY: Plenum Press, 1995.
2. Kosaric, N. & Velikonja, J. Liquid and gaseous fuels from biotechnology: challenge and opportunities. *FEMS Microbiology Reviews* **16**, 111-142 (1995).
3. Holton, W. Conard. Power Surge: Renewed Interest in Nuclear Energy. *The National Institute of Environmental Health Sciences (NIEHS)* **113**, A742-A749 (2005).
4. EcoWorld. http://www.ecoworld.com/energy/ecoworld_energy_overview1.cfm (accessed May 2007).
5. Energy Information Administration/ International Energy Outlook. <http://www.eia.doe.gov/oiaf/ieo/index.html> (accessed May 2007).
6. Atomic Alchemy: Nuclear Processes. http://library.thinkquest.org/17940/texts/fission_power/fission_power.html (accessed May 2007).
7. Lucentini, J. Using sugars, sludge, and the sea floor, can bacteria power the next green-energy alternative? *The Scientist (July)*, 43-45, 47 (2006).

8. Lovley, D.R. Taming Electricigens-How electricity-generating microbes can keep going, and going-faster. *The Scientist (July)*, 46 (2006).
9. Rabaey, K. & Verstraete, W. Microbial fuel cells: novel biotechnology for energy generation. *TRENDS in Biotechnology* **23**, 291-298. (2005).
10. Lovley, D.R. Microbial fuel cells: novel microbial physiologies and engineering approaches. *Current Opinion in Biotechnology* **17**, 327-332. (2006).
11. Lovley, D.R. Microbial Energizers: Fuel Cells That Keep on Going. *Microbe* **1**, 323-329 (2006).
12. Bond, D.R. & Lovley, D.R. Electricity Production by *Geobacter sulfurreducens* Attached to Electrodes. *Applied and Environmental Microbiology* **69**, 1548-1555 (2002).
13. Caccavo, F. Jr., Lonergan, D.J., Lovley, D.R., Davis, M., Stolz, J.F., & McInerney, M J. *Geobacter sulfurreducens* sp. Nov., a Hydrogen- and Acetate- Oxidizing Dissimilatory Metal-Reducing Microorganism. *Applied and Environmental Microbiology* **60**, 3752-3759 (1994).
14. Lovley, D.R. Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reactions in aquatic sediments. *Geochimica et Cosmochimica Acta* **52**, 2993-3003 (1988).

15. Cord-Ruwisch, R., Lovley, D.R., & Schink, B. Growth of *Geobacter sulfurreducens* with Acetate in Syntrophic Cooperation with Hydrogen-Oxidizing Anaerobic Partners. *Applied and Environmental Microbiology* **64**, 2232-2236 (1998).
16. Gregory, K.B., Bond, D.R., & Lovley, D.R. Graphite electrodes as electron donors for anaerobic respiration. *Environmental Microbiology* **6**, 596-604 (2004).
17. Bond, D. R., Holmes, D. E., Tender, L. M., & Lovley, D. R. Electrode-Reducing Microorganisms That Harvest Energy From Marine Sediments. *Science* **295**, 483-485 (2002).
18. Angenent, L.T., Karim, K., Al-Dahhen, M.H., Wrenn, B.A., & Domiguez-Espinosa, R. Production of bioenergy and biochemicals from industrial and agricultural wastewater. *TRENDS in Biotechnology* **22**, 477-485 (2004).
19. Chaudhuri, S.K. & Lovley, D.R. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. *Nature Biotechnology* **21**, 1229-1232 (2003).
20. Min, B., Kim, J.R., Oh, S., Regan, J.M., & Logan, B.E. Electricity generation from swine wastewater using microbial fuel cells. *Water Research* **39**, 4961-4968 (2005).

21. Tender, L.M., Reimers, C.E., Stecher III, H.A., Holmes, D.E., Bond, D.R., Lowy, D.A., Pilobello, K., Fertig, S.J., & Lovley, D.R. Harnessing microbially generated power on the seafloor. *Nature Biotechnology* **20**, 821-825 (2002).
22. Kim, J.R., Min, B., & Logan, B.E. Evaluation of procedures to acclimate a microbial fuel cell for electricity production. *Applied Microbiol Biotechnology* **68**, 23-30 (2005).
23. Cheng, S., Lui, H., & Logan B.E. Increased power generation in a continuous flow MFC advective flow through the porous anode and reduced electrode spacing. *Environmental Science Technology* **40**, 2426-2432 (2006).
24. Lovley, D.R. Anaerobes to the Rescue. *Science* **293**, 1444-1446 (2001).
25. Reguera, G., Nevin, K.P., Nicoll, J.S., Covalla, S.F., Woodward, T.L., & Lovley, D.R. Biofilm and Nanowire Leads to Increased Current in *Geobacter sulfurreducens* Fuel Cells. *Applied and Environmental Microbiology* **72**, 7345-7358 (2006).
26. Gregory, K.B. & Lovley, D.R. Remediation and Recovery of Uranium from Contaminated Subsurface Environments with Electrodes. *Environmental Science Technology* **39**, 8943-8947 (2005).
27. Ivy, J. Summary of Electrolytic Hydrogen Production: Milestone Completion Report. *National Renewable Energy Laboratory*, 1-27 (2004).

28. Pham, T.H., Rabaey, K., Aelterman, P., Clauwaert, P., De Schamphelaire, L., Boon, N., & Verstraete, W. Microbial Fuel Cells in Relation to Conventional Anaerobic Digestion Technology. *Engineering Life Science* **6**, 285-292 (2006).