VOLTAGE OUTPUT GENERATED BY GLUCOSE- AND ETHANOL- FED *ESCHERICHIA COLI*

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by

Jesreal L. Arcillas
Angelo M. Jamerlan
Leo Albert G. Sala
Fourth Year – Photon

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APPROVAL SHEET

This research proposal hereto entitled:

"Voltage Output Generated by Glucose- and Ethanol- fed *Escherichia coli*"

prepared and presented by Jesreal L. Arcillas, Angelo M. Jamerlan, Leo Albert G. Sala in partial fulfillment of the requirements in Science Research 2, has been examined and is recommended for acceptance and approval.

JOSETTE T. BIYO
Adviser

Approved by the Committee on Oral Examination with a grade of PASSED on February____, 2006.

ZENNIFER OBERIO
Chairman

HAROLD MEDIODIA
Member

Accepted in partial fulfillment of the requirements in Science Research 2.

DR. JOSETTE T. BIYO
OIC, Office of the Director

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ABSTRACT

Studies have shown that some bacteria are capable of producing voltage as they metabolize certain organic substances.

This study aimed to measure the voltage output generated by *E. coli* when fed with various molar concentrations of glucose (0.5, 1, 1.5, and 2) and various percent by volume concentrations of ethanol (10 %, 20 %, 30 %, and 40 %) at different periods of consumption. It also determined and compared the rate of change in the voltage output generated by *E. coli* with respect to time using glucose and ethanol.

It was hypothesized that the rates of change of voltage outputs generated when *E. coli* is fed with different glucose and ethanol concentrations do not differ significantly. The concentration of glucose and ethanol and the duration of metabolism are not good predictors of voltage output.

A galvanic cell was used to generate voltage with potassium ferricyanide at the cathode chamber and glucose- or ethanol-fed *E. coli* culture in the anode chamber. Voltage output was measured using a voltmeter from 0 to 24 hours.

Two molar glucose and 20% ethanol recorded the highest voltage output, both generated after 24 hours of consumption.

Positive rates of change with respect to time were found when *E. coli* is fed with ethanol and with 0.5M and 2M glucose. Negative rates of change were found when *E. coli* is fed 1M and 1.5M glucose.

The rate of change is significantly higher when *E.* coli is fed with 20% ethanol (α=0.05).

Multiple regression analysis revealed that concentration of glucose and ethanol and the duration of consumption of glucose are not good predictors of voltage output. However, the duration of consumption of ethanol is a good predictor of voltage output.

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LIST OF FIGURES

LIST OF TABLES

TABLES PAGE

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

A. Background of the Study

Electricity has become an important part of everyday living. Blackouts often frustrate people since electricity is very important for almost all home appliances to operate. Without it, people find inconvenience as they go about their daily routines.

The electric current and voltage, which can be derived from different possible sources, are the ones responsible for the generation of electricity. Power plants generate the necessary voltage, which in turn produce the electric current that is distributed to people for consumption. To generate voltage, power plants need a source. The most common are fossil fuels. These provide the power necessary to run an electric generator constantly.

Unfortunately, fossil fuels are irreplaceable and are depleting fast. There are alternative sources such as nuclear, geothermal, and solar energy, and this study investigates the possibility of having an equally efficient energy source for voltage generation.

Researches have shown that some bacteria have the ability to generate voltage as they metabolize their food such as glucose. *Escherichia coli* (*E. coli*) is a form of bacteria that generally live inside intestines of mammals. They become harmful once they get ingested accidentally due to food or water contamination and have been known to cause death (Wikipedia Encyclopedia, 2004).

Former researches have shown also that *E. coli* have the ability to produce discrete amounts of electricity when fed with glucose and ethanol (Anonymous, 2004).

The possibility of measuring the voltage output generated by *Escherichia coli* during glucose and ethanol digestion is the main interest of this study.

B. Statement of the Problem

The goal of this study was to determine the voltage outputs generated when *E. coli* is fed with different amounts of glucose and ethanol for 24 hours.

C. Objectives of the Study

1. To measure the voltage output (mV) generated when *E. coli* is added to various concentrations of glucose: (a) 0.5 M, (b) 1 M, (c) 1.5 M, and (d) 2 M.
2. To measure the voltage output (mV) generated when *E. coli* is added to various concentrations of ethanol: (a)10 % (b) 20 % (c) 30 %, and (d) 40 %
3. To determine the rate of change in the voltage outputs (mV) generated when *E. coli* is added to various concentrations of (a) glucose, and (b) ethanol for 24 hours.
4. To compare the rate of change in the voltage outputs generated when *E. coli* is added to various concentrations of (a) glucose, and (b) ethanol for 24 hours.
5. To identify which of these variables- (a) glucose and (b) ethanol and duration of exposure- can be a good predictor for the voltage output.

D. Hypotheses of the Study

1. There is no significant difference in the rate of change of the voltage output generated when *E. coli* is added to various concentrations of (a) glucose and (b) ethanol for 24 hours.
2. Concentration of (a) glucose and (b) ethanol and duration of exposure is not a good predictor of voltage output.

E. Significance of the Study

This study could possibly lead to the development of a new renewable energy source where it's possible to generate voltage without the extreme hazards of pollution. If *E. coli,* indeed, have the ability to generate electricity, the study could become a basis for the further development of this alternative voltage source.

The *E. coli* bacteria are very common and are very easy to culture. They grow abundantly in the intestines of warm-blooded animals and the number of *E. coli* in feces that one human passes in one day average to about 10^{11} individual bacteria. (Wikipedia, 2004). This type of voltage generation can be a possible way of generating voltage cheaply due to the abundance of *E. coli* bacteria.

E. coli are simply fed with the necessary substances to produce the amount of electricity needed. As bacteria metabolize the substances, they give out electrons (Touchette, 2003).

Since organic substances are involved, it is not deniable that CO_2 can be produced in the cell reactions but pollution can be minimized in this new way of voltage generation since only minute amounts of CO_2 are produced and are readily absorbable by autotrophs.

The results of this study will provide further enlightenment on the metabolic characteristics of *E. coli* and find potential application for this process.

F. Scope and Delimitation of the Study

This study focuses only on the generation of voltage by *E.coli* fed with various concentrations glucose and ethanol. These concentrations were purely assumed, due to the absence of literature stating exact concentrations to be utilized in the study. The amount of substrate (consisting of ethanol and glucose concentrations) used in each set-up was limited to 10 mL (0.01 L) for easier and faster digestion by *E. coli*.

Pure culture of *E. coli* was taken from the Southeast Asian Fisheries and Development Center (SEAFDEC). The specimen was cultured for only 24 hours.

Strain of *E. coli* was not identified. The growth of *E., coli* for the duration of the study was not monitored.

For the generation of voltage by E. coli, the study called for the construction of the galvanic cell. The materials comprising the said device were borrowed from the chemistry laboratory of Philippine Science High School- Western Visayas. A digital voltmeter was used in the measurement of the voltage output in each setup.

The duration of the study is limited to a maximum of 24 hours and at a minimum of five minutes. All of the studies were conducted from April to August 2005.

G. Definition of Terms

The following are the operational definitions of some terms used in the study:

- *Aseptic techniques*- are techniques used to ensure that no contamination occurs during the experiment
- *Capillary tube/tubing*- a tube of small internal diameter; holds liquid by capillary action
- *Circuit*- an electrical device that provides a path for electrical current to flow
- *Conductor*- is a kind of material that contains movable charges of electricity
- *Current*- is the rate of flow of electrons through a conductor or circuit; the unit for current is in amperes (A)
- *Digestion*- is the process whereby complex substances in food are broken down into simpler soluble compounds by enzymes
- *Electric current*- see current
- *Electric generator*- is a device that produces electrical energy from another type of energy source (mechanical energy, chemical energy, solar energy)
- *Electricity*- the manifestation of a form of energy associated with separation or movement of charged particles, especially electrons and protons
- *Electrons*- negatively charged subatomic particles
- *Erlenmeyer flask*- a conical flat-bottomed laboratory flask with a narrow neck, designed by Richard Erlenmeyer, a German chemist; widely used for culturing micro-organisms

- *Escherichia coli*- is a type of bacteria belonging to the Order Enterobacteriaceae that thrive in the intestines of warm-blooded animals
- *Ethanol*- also known as ethyl alcohol which is a term given to a class of organic compounds containing the carboxyl group, OH (hydroxide), attached to a carbon atom
- *Fossil fuels*- are any naturally occurring organic fuel formed in the Earth's crust, such as petroleum, coal and natural gas
- *Hot plate*- a portable appliance for cooking, heated formerly by a gas burner or by electrical means placed underneath it
- *Metabolized*- from metabolism, which is the sum of all chemical reactions that occur within the cells of a living organism, may it be multi-cellular or unicellular, including both formation and breakdown of complex organic compounds, eg proteins, fats, and carbohydrates
- *Nutrient broth*- a specific type of media, which is used as an artificial habitat for bacteria and other microorganisms to grow on; it is in liquid form and doesn't solidify easily
- *Potassium ferricyanide*- it is used in the cathode chamber to receive the electrons to complete the reduction process.
- *Powdered glucose*- glucose, a form of a simple sugar, in powdered form
- *Power plants*- are facilities for the generation of electric power or voltage
- *Protons*- positively charged subatomic particles.
- *psi*- (pounds per square inch) a unit of the English system for pressure

- *Salt*- a compound formed by replacing hydrogen in an acid by a metal (or a radical that acts like a metal)
- *Salt bridge*- a tube containing a saturated solution of a salt, such as potassium chloride; used to complete the electrical circuit in a galvanic cell
- *Substrate*- material or substance acted upon by an enzyme
- *Voltage*- is the force which pushes electrons through a conductor or circuit
- *Voltage output*- the amount of voltage produced by the *E. coli* as a result of the metabolism
- *Voltmeter*- a device used to measure amounts of voltage

CHAPTER II

REVIEW OF RELATED LITERATURE

Contained in this chapter are topics that propel this study such as "how bacteria can generate voltage", microbial fuel cells, galvanic cells and its parts, substrates (glucose and ethanol) and voltage output.

A. Bacteria that can generate voltage outputs

A.1 *Rhodoferax ferrireducens (R. ferrireducens)*

Studies have shown that some bacteria are capable of producing voltage as they metabolize certain substances. The *Rhodoferax ferrireducens* is a unique bacterium capable of converting sugars into electricity. It was discovered by scientists from the University of Massachusetts, led by Microbiology professor, Derek Lovley. It can transform more than 80% of a sugar grain's electron to electricity (Adams, 2004).

R. ferrireducens transfers the electrons directly into an electrode as it metabolizes sugar into electricity, with its only byproduct as carbon dioxide (CO_2) (Gawel, 2003). *Rhodoferax ferrireducens*, found on marine sediments, can generate excess electrons as part of its metabolic processes (Geobel, 2004). The bacterium was found in the mud at Oyster Bay, Virginia. It can produce enough electricity to power a cell phone battery for four days from a single sugar cube (James, 2003).

A.2 *Escherichia coli (E. coli)*

Escherichia coli are gram-negative bacilli. They grow as fermenting colonies with some beta-hemolysis upon initial isolation, are motile and non-pigmented. They belong to the Family *Enterobacteriaceae*. There are several strains of *E. coli:* Opportunistic *Escherichia coli* :enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli*(EPEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EaggEC)and uropathogenic *E. coli*(UPEC). (Univ. of Maryland)

Escherichia coli is one of the main species of bacteria that live in the lower intestines of warm-blooded animals. Its presence in ground water is a common indicator of fecal contamination. The bacteria named after its discoverer, Theodor Escherich, belongs among the Enterobacteriaceae, and is commonly used in bacterial studies.

When *E. coli* consumes sugar, energy can be harvested from chemical reactions that occur (Technology Research News, 2003).

E. coli grows at temperature range of 10 – 45 ^{O}C with optimum growth at 37 ^{O}C. it has a generation time of 0.35 hours at 40^{O}C. (Prescott, 1993)

B. The Microbial Fuel Cell (MFC): the basis of the setup

B.1 Description of the MFC

The microbial fuel cell is a device that breaks down organic matter and transforming them into energy, particularly electricity. It harnesses chemical technique similar to those the body uses to breakdown food (Biever, 2004).

Microbial fuel cells work through the action of bacteria that can pass electrons to an anode, the negative electrode of the fuel cell. The electrons then flow from the anode

through a wire, producing a current, to a cathode, the positive electrode of a fuel cell, where they combine with hydrogen ions (protons) and oxygen to form water (Cavalle, 2004).

Whereas a fuel cell operates with a battery generating electricity from a chemical reaction, a microbial fuel cell involves bacteria to metabolize the food to release electrons that yield a steady, electrical current (Kearns, 2004). A microbial fuel cell captures the hydrogen produced when microorganisms like *E. coli* metabolize carbohydrates, like sugar, in the absence of air (Technology Research News, 2003).

The naturally occurring bacteria in wastewater drive power production via a reaction that allows them to transport electrons from the cell surface to the anode. Also a reaction (oxidation) that occurs in the interior of the bacterial cell lowers the biochemical oxygen demand, which cleans the water (Cavalle, 2004).

B.2 Types of MFC's

B.2.1 According to the medium of transfer of electrons

There are two types of microbial fuel cell based on the medium of transfer of electrons. One involves the utilization of electroactive metabolites converted by microbial metabolism or enzyme reaction from the substrate, and another involves the utilization of mediators as electron transporters from a certain metabolic pathway of the microorganism or enzyme to electrodes (Halme, 1993).

A biological fuel cell is a device that directly converts biochemical energy into electricity (Halme, et al., 2004). A microbial fuel cell is a kind of biological fuel cell that can convert biochemical energy of microbes to electricity (Hyung Joo Kim, 2003).

B.2.2 According to the system of producing energy

There are three basic types of MFC's according to how they produce energy (Young et al.): (1) *depolarization cells* in which the biological system removes an electrochemical product, such as oxygen; (2) *product cells* in which an electrochemically active reactant, such as hydrogen, is biologically produced; and (3) *redox cells* in which electrochemical products are converted to reactants (ferricyanide system) by the biological system (Young et al.).

C. Galvanic cells

C.1 Description of a galvanic cell

A galvanic cell consists of at least two half cells, a reduction cell and an oxidation cell. Chemical reactions in the two half cells provide the energy for the galvanic cell operations (University of Waterloo, 2004).

Each half cell consists of an electrode and an electrolyte solution. Usually the solution contains ions derived from the electrode by oxidation or reduction reaction (University of Waterloo, 2004).

Galvanic cells generate voltage output or cell potential by Oxidation of substrate at the anode half-cell such as glucose and ethanol to form H^+ ions and CO_2. H^+ ions are then facilitated to the cathode half-cell by a salt bridge. Reduction of O_2 will then take place with H^+ ions and $Fe(CN)_6^{3-}$ as the common reducing agent to form water (University of Waterloo, 2004).

C.2 Anode half-cell (Oxidation half-cell)

The reactions in the anode chamber of the galvanic cell were the oxidation by the bacteria and the release of electrons at the anode itself. The bacteria convert the organic substrate to hydrogen ions and other by-products such as carbon dioxide (Halme, et al, 2004).

The types of chemicals that are used are specified in literatures by Park, et al, Katz, Halme, et al, and Images SI Inc. Amounts of the chemicals that will be used are not specified on most researches on galvanic cells and are considered experimental.

C.3 Cathode half-cell (Reduction half-cell)

In the cathode chamber, the hydrogen ions, which flow through a salt bridge and oxygen taken from the outside, reacted and formed water (Halme, et al, 2004).

D. Reactions inside the galvanic cells:

A. With ethanol as substrate

- Reaction at the anode (bacteria as catalyst):

$$CH_3CH_2OH + 3H_2O \rightarrow 2CO_2 + 12H^+ + 12e^-$$

- Reaction at the cathode:

$$3O_2 + 12H^+ + 12e^- \rightarrow 6H_2O$$

- Overall reaction:

$$CH_3CH_2OH + 3O_2 \rightarrow 2CO_2 + 3H_2O$$

B. With glucose as substrate

- Reaction at the anode (bacteria as catalyst):

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

- Reaction at the cathode $Fe(CN)_6^{3-}$ as reducing agent):

$$6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O$$

- Overall reaction:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

D. Glucose as an energy source

Because sugars are a substantial component of many types of waste and carbohydrate rich crops such as corn, which are a renewable energy source, carbohydrates could become economical alternatives to fossil fuels in the production of electricity (Anonymous, 2003).

Biotechnical processes with engineered strains frequently employ culture media containing glucose as the carbon and energy source. The sugar is now the most utilized raw material in industrial fermentations with *E. coli*, mostly because it is relatively inexpensive and it is the preferred carbon and energy source for this bacterium. Glucose provides carbon atoms for biomass and product generation (Microbial Cell Factories, 2005).

The maximum activity of *E. coli* is attained when fed with 1.25 molal glucose (Andersen, 1966).

Laboratory and industrial scale cultures with *E. coli* employ media containing glucose in a wide range of concentrations (Microbial Cell Factories, 2005). Furthermore, feeding strategies are usually implemented to precisely control glucose concentration through different stages in a production process. However, even under these different

scenarios, glucose will almost always be present at a concentration that can be metabolized efficiently (Microbial Cell Factories, 2005).

The overall reaction of the complete breakdown of glucose by bacteria is

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

although ethanol is formed in the intermediate processes.

The breakdown of glucose is via the Embden-Meyerof-Parnas glycolytic pathway which yields 2 moles of pyruvate, ATP and reduced nicotinamide adenine dinucleotide (NAD) per mole glucose. (Keenan)

In aerobic conditions, *E. coli* utilizes the tricarboxylic acid (TCA) cycle or Kreb's cycle and the electron transport system which oxidize pyruvate to carbon dioxide and water.(Keenan) However, it does not complete TCA cycle under anaerobic conditions or when glucose concentration is high. (Prescott, 1993)

E. Ethanol as an energy source

Ethanol is produced during the alcoholic fermentation of glucose. Pyruvate is transformed into acetaldehyde and later converted into ethanol. It is also one of the products of mixed acid fermentation of *E. coli.* About 50 μM ethanol is produced per 100 μM glucose metabolized. (Prescott, 1993)

Using ethanol with a hydrogen fuel cell can produce up to one kilowatt of power. This power is enough to supply an average home, especially those in remote areas. Some benefits of using ethanol, include reduction of dependence on imported fuels, and reduction of carbon dioxide emissions (University of Minnesota, 2004).

Ethanol can be converted easily into hydrogen using a hydrogen fuel cell, which can then be used to produce power. It is renewable, readily available and easily transportable (Farmweek.IFLB.Org, 2004).

The balanced reaction for the breakdown of ethanol by bacteria is:

$$CH_3CH_2OH + 3H_2O \rightarrow 2CO_2 + 12H^+ + 12e^-$$

F. Salt Bridges

A salt bridge, in chemistry, is a laboratory device used to connect the oxidation and reduction half-cells of a galvanic cell (electrochemical cell). They are usually classified into two types. glass tube and filter paper (Wikipedia, 2005). In the study, a glass tube salt bridge is used.

A glass tube salt bridge usually consists of U-shaped glass tubes filled with a relatively inert electrolyte, such as potassium chloride or sodium chloride (Wikipedia, 2005).

The conductivity of glass tube bridges depend mostly on the concentration of the electrolyte solution. An increase in the concentration increases conductivity (Wikipedia, 2005).

A salt bridge is an important component of a galvanic cell. The salt bridge exists to provide the electrical connection between the two half-cells while keeping the two reactions separate. The salt bridge allows the electron transfer between the two vessels (Salt Institute. 2000).

G. Voltage

Voltage is the measure of electric potential energy per unit charge and is measured in joules per coulomb or volts (HyperPhysics, 2005). Electric potential energy per unit time is electricity, measured in watts. Thus, the electric power produced is directly proportional to the voltage (Wikipedia, 2005).

Voltage is also given by the equation:

$V = IR$

V is the voltage in volts (V), I is the current in amperes (A) and R is the resistance in ohms (Ω). Current is the no. of charges that pass through a given area per unit time. Thus the greater the current, the faster the charges pass through the area. When the charges flow fast, the kinetic energy also increases. Since power is measured through kinetic energy per unit time, an increase in the current would suggest an increase in the electric power. Current is dependent on the voltage, so, voltage is directly proportional to the electric power (Wikipedia, 2005).

CHAPTER III

METHODOLOGY

A. Materials

One Pure Culture of *Escherichia coli*

45 g Glucose

270mL 99.5%v Ethanol Solution

27 Test tubes

3.90g Nutrient Broth

35 grams sodium chloride

540mL 0.02 M potassium hexacyanoferrate or potassium ferricyanide (K_3 [Fe $(CN)_6$])

One 200mL Erlenmeyer flask

Graduated cylinder

54 25 mL Erlenmeyer flasks

9 g aluminum foil

27 capillary tubes

Alcohol lamp

Pipette

Stirring rod

Incubator

Voltmeter

Autoclave

Inoculating loop

Distilled water

Gathering of Materials

Escherichia coli, the research specimen, was obtained from the South East Asian Fisheries and Development Center (SEAFDEC).

All needed laboratory equipment, apparatus and chemicals were obtained from the Philippine Science High School-Western Campus Research and Chemistry laboratories.

Other materials unavailable from the aforementioned agencies were purchased from companies, stores or agencies legally selling them.

B. Research Design

This study aimed to measure the different voltage outputs generated by *Escherichia coli* when fed with different concentrations of glucose and ethanol. It also aimed to compare the rate of change of voltage output generated when *E. coli* is fed with glucose and ethanol concentrations. It further aims to determine if the glucose and ethanol concentrations are good predictors of voltage increase per unit time.

It is hypothesized that the rates of change of voltage outputs generated by *Escherichia coli* when fed with different concentrations of glucose and ethanol do not differ significantly.

The researchers devised and used a galvanic cell which generates voltage by oxidation at its anode half-cell and reduction at its cathode half-cell. The oxidation

process is the oxidation of glucose and ethanol catalyzed by bacteria and the reduction process is the reduction of oxygen reduced by potassium ferricyanide.

Voltage output was tested immediately after covering the galvanic cell and after 0 hours, five minutes, one hour,2 hours 3 hours four hours, five hours, 6 hours, seven hours, eight hours, 15 hours, 20 hours and 24 hours. Three replicates were made for every set-up. Incubation of galvanic cell was not needed since it operates best at room temperature.

All other factors are maintained constant.

C. General Procedures

Sterilization of Glassware

All glassware that was used was thoroughly washed with soap and water. Glassware was autoclaved at 15 psi (121°C) for 15-30 minutes (in order for formerly existing contaminants on these materials to be eradicated).

Preparation of Culture Media

Nutrient Broth culture was prepared for *E. coli* by first weighing 3.90g unhydrated Nutrient Broth on an analytical balance. Then the solid broth was dissolved in 300mL distilled water in an Erlenmeyer flask and was autoclaved for 15-30 minutes. Ten milliliters of liquid culture medium was transferred in each 27 test tubes using a sterilized

pipette. Then, each test tube was labeled with the different ethanol and glucose concentrations.

Inoculation of Pure Culture

The wire of the inoculating loop was heated until red-hot in an approximately 80° inclination with respect to the flame of the alcohol lamp and was cooled for 15-30 seconds after heating. *Escherichia coli* culture was scooped from its former container using the inoculating loop and was transferred inside the test tubes containing the prepared culture medium. The bacterial culture was then incubated at 37°C for 24 hours (Kennesaw State University, 1999).

Preparation of Glucose Solutions

Glucose solutions in 0.5 M, 1 M, 1.5 M and 2 M 15mL each were used. In obtaining 0.5 M glucose solution, 1.35g glucose was added with distilled water until the 15ml mark is reached; in 1 M, 2.7g; in 1.5 M, 4.05g; and in 2 M, 5.4g each added with distilled water until the 15ml mark is reached.

Preparation of Ethanol Solutions

Thirty milliliters of each ethanol concentrations were prepared with the ratio of volume of water and 99.5% ethanol shown in Table 1.

Table 1. Corresponding volumes of water and 99.5% ethanol with the desired ethanol concentrations.

ETHANOL CONCENTRATION (%v)	VOLUME OF H_2O (mL)	VOLUME OF 99.5% ETHANOL (mL)
10	26.98	3.02
20	23.97	6.03
30	20.95	9.05
40	17.94	12.06

Preparation of Potassium ferricyanide Solution

Five hundred and forty milliliters of 0.02 M potassium ferricyanide was prepared by dissolving 3.442 grams of solid potassium ferricyanide in distilled water until the solution becomes 540mL.

Preparation of Saturated Salt Solution

A saturated NaCL solution was prepared for the salt-bridge. Ten milliliters of saturated NaCL solution by dissolving 3.5 grams of solid NaCL with 10mL distilled water.

Construction of galvanic cell and preparation of the salt bridge

Materials:

54 25mL Erlenmeyer flasks

27 capillary tubes

9.00g Aluminum foil

540mL 0.02M potassium hexacyanoferrate or potassium ferricyanide

27 galvanic cells

The prepared glucose and ethanol solutions

Escherichia coli liquid culture

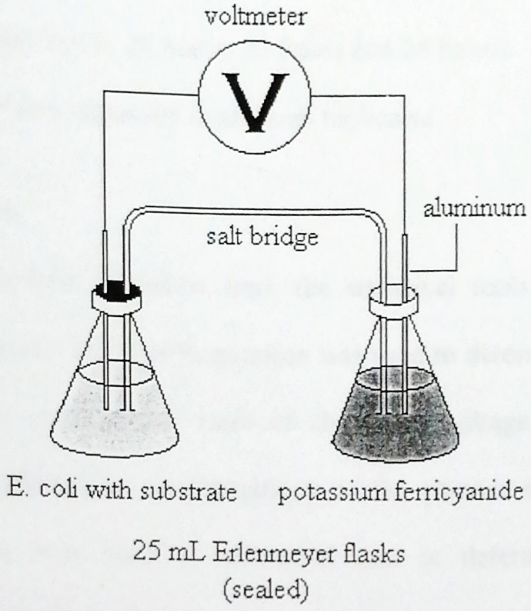
The galvanic half-cell was assembled in the manner shown in Figure 1. Twenty-seven capillary tubes were bended in a U-form. 0.3 grams of aluminum at each side of the half-cell were placed as extensions of the voltmeter electrodes to avoid contamination of voltmeter electrodes.

The anode chamber was filled with 10mL organic substrate (the different glucose or ethanol concentrations) and 10mL *Escherichia coli* in culture medium (Katz, 2000).

The cathode chamber was filled with 20mL 0.02M potassium hexacyanoferrate or potassium ferricyanide (Images SI Inc and Park, et al).

The capillary tubes were filled with saturated NaCl solution and installed according to the orientation shown in Figure 1.

Figure 1. The galvanic cell.



Twenty-seven galvanic cells were constructed following the above design.

Testing of Voltage Output

Materials:

Voltmeter

Prepared galvanic cells

The voltage output produced by the galvanic cell was tested using a voltmeter by connecting the voltmeter electrodes with the matching galvanic cell aluminum electrodes according to charge.

Voltage output was tested immediately after covering the galvanic half-cells and was tested again after five minutes, one hour, 2 hours 3 hours four hours, five hours, 6 hours, seven hours, eight hours, 15 hours, 20 hours and 24 hours.

Voltage output was measured in all three replicates.

D. Statistical Analysis

Mean and Standard Deviation were the statistical tools used to compare the voltage outputs measured. Multiple Regression was used to determine the rate of change of voltage output per concentration rates of change of voltage outputs generated by *Escherichia coli* as affected by concentrations of the glucose and ethanol. One-Way Analysis of Variance was used as inferential tool in determining the significant differences in the rates of change of voltage outputs generated by *Escherichia coli* as affected by a) concentrations of the substrates used and b) duration of exposure of substrates to *Escherichia coli*.

All statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 7.5.

E. Handling and Disposal

Some microbiological Practices

Access to the laboratory was limited or restricted at the discretion of the laboratory director when experiments were in progress.

The researchers washed their hands after they handled viable materials, after removing gloves, and before leaving the laboratory.

Eating, drinking, and handling contact lenses were not permitted in the work areas.

All procedures were performed carefully to minimize creation of splashes or aerosols.

The wire of the inoculating loop was heated before and after contact with the microorganism.

The mouth of the flask and each test tube were always heated before and after the test tubes are opened and were immediately covered with cotton plugs.

All cultures and stocks were decontaminated with a commercial disinfectant before autoclaving.

Cultures and potentially infectious wastes were placed in a container with a cover that prevents leakage during collection, handling, processing, and storage.

Laboratory equipment and work surfaces were decontaminated with an effective disinfectant on a routine basis, after work with infectious materials.

CHAPTER IV

RESULTS AND DISCUSSION

The study made use of a galvanic cell which can generate voltage from *E. coli* when fed with the different concentrations of glucose and ethanol. It specifically measured the voltage output generated when *E. coli* is fed with various concentrations of glucose: 0.5 M glucose, 1 M glucose, 1.5 M glucose, and 2 M glucose. It also measured the voltage output generated when *E. coli* is fed with various concentrations of ethanol: 50% alcohol, 75% alcohol, 80% alcohol, and 90% alcohol. It measured the voltage output generated in different concentrations of glucose and ethanol at various duration of exposure to *E. coli*: 0 hours, 0.833 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 15 hours, 20 hours, and 24 hours.

It is hypothesized that the rate of change of voltage outputs generated when *E. coli* is fed with increasing concentrations of glucose and ethanol at various duration of exposure does not differ significantly. It is also hypothesized that the concentrations of (a) glucose and (b) ethanol and duration of exposure are not good predictors of voltage output.

Results

Workability of the Galvanic Cell

A preliminary test was done to determine if the galvanic cell can generate voltage or not. Results revealed that the galvanic cell without glucose and ethanol and set-ups without bacteria can not generate voltage.

Voltage Output Generated by *E. coli* at Different Time Intervals

a. When Fed with Glucose

After various glucose concentrations were fed to *E. coli*, the voltage output generated by *E. coli* in millivolts (mV) at different time intervals was measured.

It was observed that the voltage output generated by *E. coli* when fed with glucose concentrations at different time intervals fluctuates.

The highest mean voltage output generated by glucose-fed *E. coli* was 445.993mV, generated after 24 hours of exposure to 2M glucose. The lowest mean voltage output generated was 17.53 mV, generated five minutes after consumption of 0.5 M glucose.

Results showed that the highest mean voltage output generated by *E. coli* when fed with 0.5M glucose was 319.9 mV after 20 hours of substrate consumption and lowest mean voltage output was 17.53 mV and was generated after five minutes of consumption.

Results further showed that when *E. coli* is fed with 1M glucose, the highest generated mean voltage is 452.667mV which was achieved after two hours of

consumption and the lowest mean voltage was 40.9mV which was generated after eight hours of consumption.

E. coli generated its highest mean voltage (391.667mV) when fed with 1.5M glucose after two hours of consumption and achieved its lowest (34.33mV) after 5 hours of consumption.

Results revealed that the highest mean voltage generated by *E. coli* after being fed with 2M glucose was 445.933mV that was generated after 24 hours of consumption and the lowest was 89.4mV after three hours of consumption .

The voltage output peaked earlier when *E. coli* is fed with 1M and 1.5M glucose and decrease thereafter. The voltage output generated when *E. coli* is fed with 0.5M and 2M peaked at the later stage of glucose consumption.

Data is presented in Figure 2.

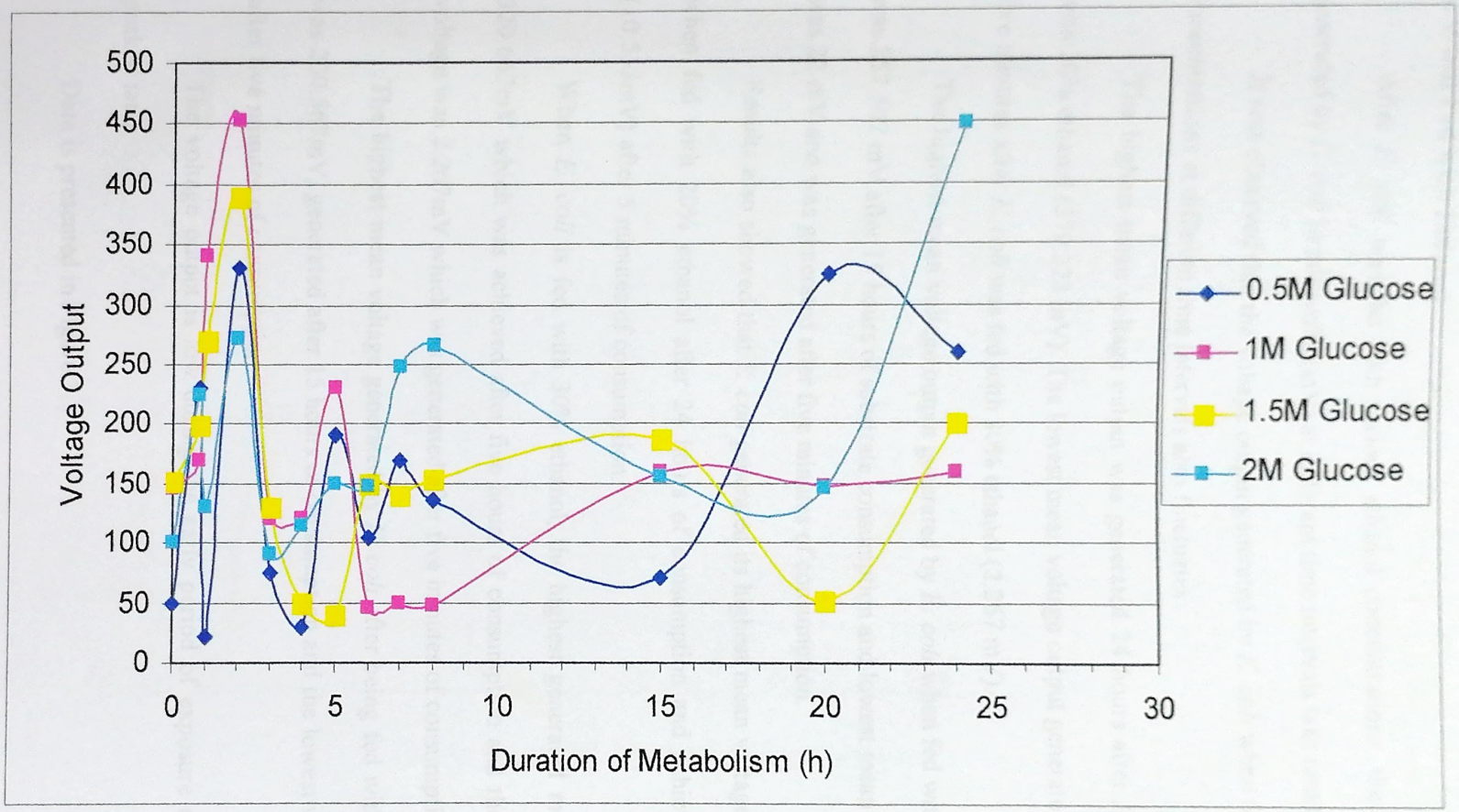


Figure 2. Mean voltage output at different time intervals generated when *E. coli* is fed with different concentrations of glucose.

b. When Fed with Ethanol

After *E. coli* was fed with various ethanol concentrations, the voltage output generated by *E. coli* in millivolts (mV) at different time intervals was measured.

It was observed that the voltage output generated by *E. coli* when fed with ethanol concentrations at different time intervals also fluctuates.

The highest mean voltage output was generated 24 hours after *E. coli* was fed with 20% ethanol (379.223 mV). The lowest mean voltage output generated was recorded five minutes after *E. coli* was fed with 30% ethanol (2.267 mV).

The highest mean voltage output generated by *E. coli* when fed with 10% ethanol was 257.567 mV after 15 hours of substrate consumption and lowest mean voltage output was 22 mV and was generated after five minutes of consumption.

Results also showed that *E. coli* generated its highest mean voltage (379.233mV) when fed with 20% ethanol after 24 hours of consumption and achieved its lowest (10.533mV) after 5 minutes of consumption.

When *E. coli* is fed with 30% ethanol, the highest generated mean voltage is 320.667mV which was achieved after five hours of consumption and the lowest mean voltage was 2.267mV which was generated after five minutes of consumption.

The highest mean voltage generated by *E. coli* after being fed with 40% ethanol was 200.567mV, generated after 15 hours of consumption and the lowest was 13.333mV after five minutes of consumption.

The voltage output is low during the early period of exposure to ethanol and peaked later.

Data is presented in Figure 3.

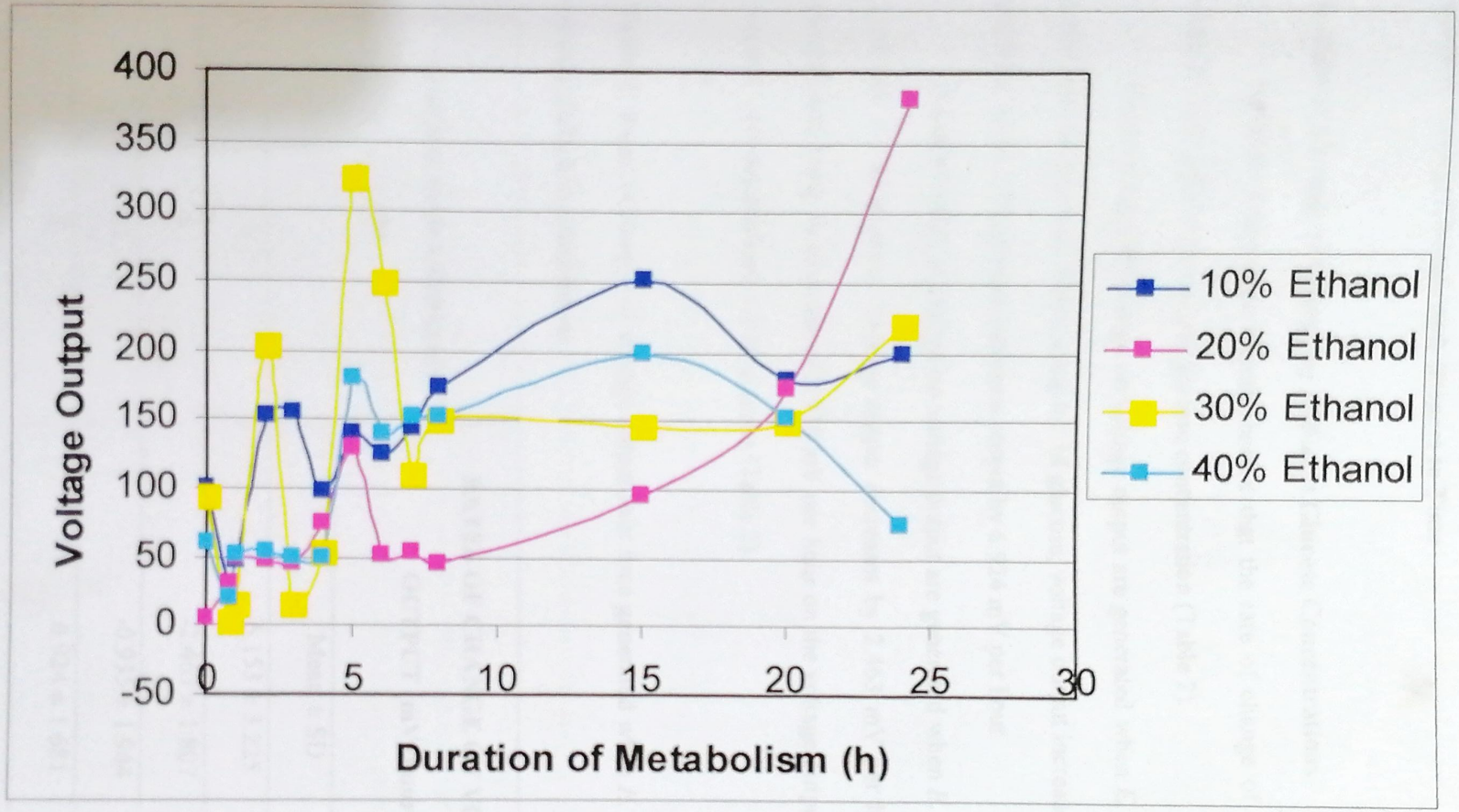


Figure 3. Mean voltage output at different time intervals generated when *E. coli* is fed with different concentrations of ethanol.

Rates of Voltage Change with Respect to Time

A. Rate of Change of Voltage by Different Glucose Concentrations

Multiple Regression results showed that the rate of change of voltage outputs when *E. coli* is fed with various glucose concentration (Table 2).

Positive rates of change on voltage output are generated when *E. coli* is fed with 0.5M and 2M glucose. When using 0.5 M glucose, voltage output increases by 6.153 mV per hour while 2 M glucose increases output by 6.924 mV per hour.

Negative rates of change on voltage output are generated when *E. coli* is fed with 1 M and 1.5 M glucose. Voltage output decreases by 2.463 mV per hour when 1 M glucose was used. A decrease of 0.935 mV per hour on the voltage output was observed when *E. coli* metabolized 1.5 M glucose (Table 2).

Table 2. Rates of change of voltage output over time generated when *E. coli* is fed with various glucose concentrations.

GLUCOSE CONCENTRATION (M)	**RATES OF CHANGE OF VOLTAGE OUTPUT (mV /hour)**
	Mean ± SD
0.5	6.153 ± 3.225
1	-2.463 ± 1.807
1.5	-0.935 ± 1.944
2	6.924 ± 1.681

One-Way Analysis of Variance shows that there is a significant difference among the rates of changes of voltage output generated when *E. coli* is fed with different glucose concentrations (Table 3).

Table 3. One-Way Analysis of Variance of the rates of change of voltage output caused when *E. coli* is fed with various glucose concentrations ($p=0.002$, $\alpha=0.05$).

	SUM OF SQUARES	df	MEAN SQUARE	F	Sig.	Interpretation
Between Groups	207.959	3	69.320	13.675	.002	Significant
Within Groups	40.554	8	5.069			
Total	248.513	11				

* The mean difference is significant at the .05 level.

Post Hoc Analysis showed that the rate of change of voltage output with respect to time using 0.5M and 2M glucose concentrations, both having positive rates, do not differ significantly. No significant difference was also observed with the rates of change of voltage output with respect to time using 1M and 1.5M glucose concentrations, both having negative rates (Table 4).

Table 4. Post Hoc test of the significant difference between the individual rates of change of voltage output when *E. coli* is fed with increasing concentrations of glucose as time increases.

GLUCOSE CONC.	GLUCOSE CONC.	Mean Difference	Std. Error	Sig.	Interpretations
0.5M	2M	-.771000	1.838	.980	Not significant
1M	1.5M	-1.528200	1.838	.873	Not significant

* The mean difference is significant at the .05 level.

B. Rate of Change of Voltage by Different Ethanol Concentrations

The Multiple Regression results show the rates of change of voltage outputs generated by *E. coli* when fed with various ethanol concentrations (Table 5).

All concentrations of ethanol show positive rates of change in the voltage output generated by *E. coli* in 24-hour period.

The highest rate of change was 9.660 mV/hour, generated when 20% ethanol was fed to *E. coli*. The lowest rate was 2.712 mV/hour, generated by *E. coli* fed with 40% ethanol (Table 5).

Table 5. Rates of change of voltage output over time generated when *E. coli* is fed with various ethanol concentrations.

ETHANOL CONCENTRATION (%V)	RATES OF CHANGE OF VOLTAGE OUTPUT (mV/hour)
	Mean ± SD
10	5.532 ± 2.860
20	9.660 ± 1.358
30	3.237 ± 2.278
40	2.712 ± 1.490

* The mean difference is significant at the .05 level.

One-Way Analysis of Variance shows that there is a significant difference among the rates of changes of voltage output generated when *E. coli* is fed with different ethanol concentrations (Table 6).

Table 6. One-Way Analysis of Variance of the rates of change of voltage output caused when *E. coli* is fed with increasing ethanol concentrations.

	SUM OF SQUARES	df	MEAN SQUARE	F	Sig.	Interpretation
Between Groups	90.048	3	30.016	6.887	0.013	Significant
Within Groups	34.868	8	4.359			
Total	124.916	11				

* The mean difference is significant at the .05 level.

Post Hoc Analysis shows that the rate of change of voltage generated by *E. coli* when fed with 10%, 20%, 30% and 40% ethanol has the relationship

$$R_{20\%} > R_{10\%} = R_{30\%} = R_{40\%}$$

The rate of change of the voltage output with respect to time is significantly higher when *E. coli* is fed with 20% ethanol than with any other concentrations of ethanol (Table 7).

Table 7. Post Hoc Analysis of the significant difference between the rates of change of voltage output when *E. coli* is fed with various concentrations of ethanol for 24 hours.

ETHANOL CONC.	ETHANOL CONC.	Mean Difference	Std. Error	Sig.	Interpretations
10%	20%	-4.127667	1.705	.199	Not significant
10%	30%	2.295667	1.705	.630	Not significant
10%	40%	2.820000	1.705	.477	Not significant
20%	30%	6.423333	1.705	.035	Significant
20%	40%	6.947667	1.705	.024	Significant
30%	40%	.524333	1.705	.992	Not significant

- The mean difference is significant at the .05 level.

Glucose Concentration and Duration of Exposure to Glucose as Predictors of Voltage Output

Multiple Regression was conducted with the hypothesis that the rate of change of the voltage output with respect to time is equal to 0 or is constant. If the rate is significantly higher than 0, the variable can be used as a predictor of the voltage output.

Results showed that when glucose concentrations are used, both glucose concentration and duration of exposure to glucose are poor predictors of voltage change. Voltage output is independent of glucose concentration and duration of exposure (Table 8).

Table 8. Multiple Regression results of two independent variables namely, time and glucose concentrations with voltage as dependent variable.

Independent Variable	Rate of Change Caused on Voltage (mV/ h)	t-value	Sig.	Interpretation
Duration of Exposure	2.420	1.907	.058	Poor Predictor of Voltage Output
Glucose Concentration	3.311	.197	.844	Poor Predictor of Voltage Output

* The mean difference is significant at the .05 level.

Ethanol Concentration and Duration of Exposure to Ethanol as Predictors of Voltage Output

Multiple regression results showed that when *E. coli* is fed with ethanol, the rate of voltage change is not significantly different from zero, thus ethanol concentration is a poor predictor of voltage output.

The duration of exposure to ethanol caused a rate of voltage change that is significantly different from zero thus it is a good predictor of voltage output. (Table 9).

Table 9. Multiple Regression results of two independent variables namely, time and ethanol concentrations with voltage as dependent variable.

Independent Variable	Rate of Change Caused on Voltage (mV/ h)	t-value	Sig.	Interpretation
Duration of Exposure	5.285	6.609	.000	Good Predictor of Voltage Change
Ethanol Concentration	-.111	-.210	.834	Poor Predictor of Voltage Change

* The mean difference is significant at the .05 level.

Discussion

Production of Voltage by *E. coli* when Fed with Glucose and Ethanol

Generally, the galvanic cell produces voltage by the oxidation that takes place at its anode chamber and the reduction that takes place in the cathode chamber. In this study the oxidation process that took place is the breaking down of ethanol and glucose to carbon dioxide, hydrogen ions and electrons while the reduction process is the reduction of oxygen by the reducing agent-potassium ferricyanide to form water. Since both oxidation and reduction reactions in the two chambers are fulfilled the galvanic cell produced voltage.

Results showed that the galvanic cell set-ups that lack bacteria do not produce voltage. This is because the oxidation process in the anode half-cell needs bacteria to oxidize it and to spontaneously release H^+ ions and electrons.

Also, the galvanic cell set-ups lacking substrate (glucose/ethanol) do not produce voltage because there are no sources of ions and the bacteria has nothing to oxidize.

Results showed that the voltage readings caused by glucose concentrations is higher than that of voltage readings of ethanol concentrations.

Comparing the reactions in the anode chamber with glucose and ethanol as substrate, a mole of ethanol can only produce 12 moles of H^+ and 12 moles of e^- while one mole of glucose produces 24 moles of H^+ and 24 moles of e^-. Since voltage is directly proportional to the amount of charges (electrons) present, set-ups with glucose as substrate will definitely produce higher voltage output.

Implication of Voltage

When a galvanic cell produces voltage, if it were connected to a circuit, it will provide electric current and eventually electric power. It is a potential source of electricity.

A normal small dry cell could produce 1.5V while the galvanic cell in this study could produce a maximum of about 453mV thus, about 4 galvanic cells similar to this study could also function like that of a single 1.5V dry cell.

Rate of Change of Voltage

When *E. coli* is fed with glucose concentrations, results showed that there is a significant difference in the rates of change of voltage output generated. This means that varying concentrations of glucose, when fed to *E. coli*, produced significantly different rates of change of voltage outputs.

According to Andersen (1966) *E. coli* attains its maximal activity when fed with 1.25m glucose which is equal to 1M glucose. When *E. coli* is exposed to an environment ambient for active metabolism, it requires more source of energy thus glucose breakdown is faster. This could be the reason why the voltage output peaks earlier when 1M and 1.5M glucose was fed to *E. coli.* With this more glucose is metabolized earlier reducing the concentration of glucose as time passes. This could have led to negative rates of change of voltage.

The rate of voltage caused by 0.5 M is not significantly different with that of the rate of change caused by 2M glucose. When *E. coli* is exposed to an environment where source of carbon is scarce or is very much higher than the normal, it adapts to the

environment by either utilizing other source of carbon available or using other metabolic pathway to breakdown glucose. In aerobic conditions, *E. coli* utilizes the tricarboxylic acid (TCA) cycle or Kreb's cycle. (Keenan) However, it does not complete TCA cycle under anaerobic conditions or when glucose concentration is high. (Prescott, 1993)

With this, breakdown of glucose or release of ions is lower at the early stage of consumption but increases as the duration of exposure increases. This action causes a positive rate of change to the voltage output.

When *E. coli* is fed with ethanol concentrations, results also showed that there is a significant difference in the rates of change of voltage output generated. This means that varying concentrations of ethanol, when fed to *E. coli,* produced significantly different rates of change of voltage outputs. Statistical analysis showed that all rates of change of voltage generated by ethanol fed *E. coli* are all positive.

Glucose is the main carbon source of *E. coli*, thus it is the ingredient for most *E. coli* media. In the absence of glucose, *E. coli* utilizes other carbon source. One of them is ethanol. Ethanol is produced during the alcoholic fermentation of glucose, an alternative pathway aside from the tricarboxylic acid (TCA) pathway.

Since glucose is relatively low in the solution where ethanol is added, *E. coli* adapts to the environment thus metabolism or activity is low during the early stage of exposure to ethanol. With this, breakdown of ethanol is low at the early stage generating lower voltage output. Activity increases with time thus a positive rate of change was observed when *E. coli* is fed with ethanol.

Glucose and Ethanol and the Duration of Consumption as Predictors of Voltage Output

With the hypothesis that the rate of change in voltage output generated by *E. coli* fed with different glucose and ethanol concentration with respect to time is equal to zero or is constant, a multiple regression analysis was performed. If the rate of change in voltage caused by each variable is significantly different from zero, then that variable is considered a good predictor of voltage output.

When *E. coli* is fed with glucose concentrations, the rate of change in voltage caused by glucose concentration and the duration to which glucose is consumed were not significantly different from zero therefore they are good predictors of voltage output. This is because the maximal activity of *E. coli* is not proportional to time and concentration. *E. coli* acts better at certain concentrations only. Maximum activity and maximum voltage output can only be generated when *E. coli* is fed with specific amount of glucose only at a certain duration of exposure.

Also, the rate of voltage change caused by ethanol concentration is not significantly different from zero thus ethanol concentration is not a good predictor of voltage output. This is because the maximal activity of *E. coli* is only at certain concentrations of carbon source. If it is lower or higher than the optimum requirement, activity of the organism is affected.

The rate of voltage change caused by the duration of ethanol consumption is significantly different from zero thus it is a good predictor of voltage output. Since the rate of change on voltage output is positive with respect to time when ethanol is used, an increase in the duration of exposure will increase the voltage output.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

In this study, the researchers measured the voltage output and compared the rates of change of voltage output generated by *E. coli* when fed with variuos glucose and ethanol concentrations at different durations of metabolism using a galvanic cell.

It specifically aimed to:

1. measure the voltage output generated when *E. coli* is added to various concentrations of glucose: (a) 0.5 M, (b) 1 M, (c) 1.5 M, and (d) 2 M.
2. measure the voltage output generated when *E. coli* is added to various concentrations of ethanol: (a) 10 % ,(b) 20 %, (c) 30 %, and (d) 40 %
3. determine the rate of change in the voltage outputs generated when *E. coli* is added to various concentrations of (a) glucose, and (b) ethanol for 24 hours.
4. compare the rate of change in the voltage outputs generated when *E. coli* is added to various concentrations of (a) glucose, and (b) ethanol for 24 hours.
5. identify whether concentration of (a) glucose and (b) ethanol and duration of exposure can be a good predictor for the voltage output.

It is hypothesized that the rates of change of voltage outputs generated when *E. coli* is fed with glucose and ethanol concentrations do not differ significantly. It is also hypothesized that the concentration of glucose and ethanol and the duration of metabolism are not good predictors of voltage output.

A. Summary of Results

Results showed that:

1. Voltage cannot be generated without substrate or bacteria.
2. Highest voltage output can be generated when *E.* coli is fed with 2M glucose and 20% ethanol both after 24 hours of consumption.
3. There are positive rates of change of voltage output generated by *E. coli* when fed with 10%, 20%, 30% and 40% ethanol.
4. The rate of change in the voltage output with respect to time is significantly higher when 20% ethanol is used.
5. The voltage output peaks earlier when 1M and 1.5M glucose is used. There are negative rates of change of voltage output generated by *E. coli* when fed with 1M and 1.5M glucose.
6. There are positive rates of change of voltage output generated by *E. coli* when fed with 0.5M and 2M ethanol.
7. The rates of change in the voltage output with respect to time when 1M and 1.5M glucose is used are statistically equal.
8. The rates of change in the voltage output with respect to time when 0.5M and 2M glucose is used are statistically equal.
9. When glucose is used, the concentration and duration of exposure are not good predictors of voltage output.
10. When ethanol is used, the concentration is not a good predictor of voltage output. However, the duration of exposure is a good predictor of voltage output.

B. Conclusions

Voltage can be generated by a galvanic cell that has its oxidation process as the metabolism of glucose and ethanol by *E. coli*.

Concentration of glucose and ethanol and the duration of exposure to glucose affect the voltage output. However, the relationship derived could not be used to make inferences about the relationship between the variables beyond the range of values of the variables used in the study.

The duration of exposure to ethanol is a good predictor of voltage output. Hence, as the elapsed time increases, voltage output increases.

C. Recommendations

The researchers recommend this research paper as a reference for future studies regarding the generation of electricity by microorganisms especially, *E. coli.*

For similar studies, the researchers also recommend:

- the use of other microorganisms to produce electricity in comparison to the production of electricity by *E. coli;*
- the usage of computer-linked voltmeters to record voltage readings from time 0 up to the desired time interval.
- the feeding of other substrates such as carbohydrates and other simple sugars and alcohols to *E. coli* to produce electricity.
- monitor the rate of growth of *E. coli* to compare it with the rate of change in voltage output.

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Appendix

Raw Data

1st Trial at 0.5 M glucose	
Time (h)	Voltage (mV)
0	105
0.0833	49.4
1	30.2
2	144.6
3	211
4	80.7
5	131
6	139.9
7	131.4
8	144.6
15	135.6
20	137
24	167.8

2nd Trial at 0.5 M glucose	
Time (h)	Voltage (mV)
0	30
0.0833	114.5
1	50
2	50
3	31
4	75
5	102
6	47.4
7	50.4
8	45
15	109
20	209
24	289.5

3rd Trial at 0.5 M glucose	
Time (h)	Voltage (mV)
0	106
0.0833	123
1	24.5
2	300
3	22
4	88.7
5	369
6	309
7	139
8	162
15	170
20	187.6
24	152.6

1st Trial at 1.0 M glucose	
Time (h)	Voltage (mV)
0	55.3
0.0833	115
1	44.6
2	62
3	79
4	62.6
5	137.3
6	74.5
7	219
8	167
15	205
20	221
24	79

2nd Trial at 1.0 M glucose	
Time (h)	Voltage (mV)
0	82
0.0833	64
1	44
2	136.9
3	135.7
4	100.3
5	139
6	68
7	134.2
8	212
15	321
20	164.6
24	160.5

3rd Trial at 1.0 M glucose	
Time (h)	Voltage (mV)
0	26
0.0833	126.7
1	48
2	29.1
3	27
4	69.8
5	116.6
6	59
7	158.7
8	32.6
15	83.8
20	137.9
24	390.7

1st Trial at 1.5 M glucose	
Time (h)	Voltage (mV)
0	110
0.0833	191
1	12
2	213.3
3	19.3
4	42.6
5	341
6	203.6
7	99.2
8	131
15	151.5
20	97.4
24	222.5

2nd Trial at 1.5 M glucose	
Time (h)	Voltage (mV)
0	75
0.0833	93.4
1	68
2	45.8
3	31.6
4	44.1
5	250.6
6	159.7
7	56.7
8	142
15	195.8
20	128.8
24	34.4

3rd Trial at 1.5 M glucose	
Time (h)	Voltage (mV)
0	104.7
0.0833	63
1	55.4
2	186
3	144.7
4	100.7
5	135
6	142
7	153
8	166
15	314.9
20	233
24	271

1st Trial at 2.0 M glucose	
Time (h)	Voltage (mV)
0	14.3
0.0833	171
1	44.5
2	34.8
3	16.2
4	67
5	155
6	64
7	68
8	40
15	72.8
20	177
24	458

2nd Trial at 2.0 M glucose	
Time (h)	Voltage (mV)
0	52.9
0.0833	151.5
1	25
2	106.8
3	14
4	75
5	252
6	228
7	137
8	159
15	104.7
20	164
24	276

3rd Trial at 2.0 M glucose	
Time (h)	Voltage (mV)
0	72
0.0833	100.3
1	46.2
2	70
3	27
4	42.7
5	154
6	189.5
7	192.7
8	162
15	200.9
20	120.9
24	100

1st Trial at 10% ethanol	
Time (h)	Voltage (mV)
0	20
0.0833	203
1	12.6
2	208.4
3	77.2
4	39.5
5	198
6	117
7	219
8	128
15	56.5
20	442
24	228.2

2nd Trial at 10% ethanol	
Time (h)	Voltage (mV)
0	11
0.0833	222
1	400
2	434
3	130
4	120.8
5	128
6	30
7	52.8
8	55
15	173.7
20	108
24	138.2

3rd Trial at 10% ethanol	
Time (h)	Voltage (mV)
0	2.3
0.0833	160.7
1	221.7
2	400
3	115.6
4	60.6
5	43.1
6	146
7	145.7
8	189
15	182
20	40
24	236

1st Trial at 20% ethanol	
Time (h)	Voltage (mV)
0	14.2
0.0833	190.3
1	171.5
2	367
3	79
4	152.6
5	120
6	155
7	210.2
8	249.5
15	195
20	130.6
24	455.2

2nd Trial at 20% ethanol	
Time (h)	Voltage (mV)
0	24
0.0833	225.1
1	14
2	300
3	82
4	23
5	237
6	70.6
7	56.7
8	131.5
15	80
20	131.7
24	236.6

3rd Trial at 20% ethanol	
Time (h)	Voltage (mV)
0	15
0.0833	181.6
1	377
2	519
3	127
4	150.6
5	274.6
6	32
7	54
8	22
15	190
20	182
24	142

1st Trial at 30% ethanol	
Time (h)	Voltage (mV)
0	2
0.0833	200
1	319
2	398
3	141
4	47.6
5	30.9
6	139
7	93.9
8	176
15	190
20	67
24	254

2nd Trial at 30% ethanol	
Time (h)	Voltage (mV)
0	7
0.0833	218.7
1	180
2	340.6
3	101
4	99.6
5	154.2
6	157.5
7	278
8	231.7
15	133.8
20	155
24	533

3rd Trial at 30% ethanol	
Time (h)	Voltage (mV)
0	22
0.0833	257
1	26
2	450
3	67.5
4	27
5	142.4
6	125
7	192.7
8	138
15	85
20	386
24	313

1st Trial at 40% ethanol	
Time (h)	Voltage (mV)
0	5.6
0.0833	123
1	329.2
2	405
3	93
4	72
5	286
6	21
7	26
8	45.7
15	126.4
20	139
24	205

2nd Trial at 40% ethanol	
Time (h)	Voltage (mV)
0	2.5
0.0833	216
1	286
2	377
3	121.3
4	44
5	28.4
6	153.6
7	165
8	106
15	189
20	53.7
24	115

3rd Trial at 40% ethanol	
Time (h)	Voltage (mV)
0	18.8
0.0833	260
1	156.1
2	134
3	88.2
4	94.6
5	180.3
6	98.1
7	238
8	324
15	141.4
20	123
24	349.6