Chapter 3

Development Of MFC Based Bio Dry Cell Technology

3.1 Introduction

As with most biological systems, it is desirable to identify the microorganisms present in the anodic chambers of microbial fuel cells (MFCs). Unfortunately, the identification of each type of microorganism present would be both resources intensive and time-consuming. Most of the bacteriasrelease electrons and protons in the method of metabolizing organic waste. The aforementioned fact is that irrespective of the growth and incubation conditions prevalent. These electrons and protons may be harnessed using appropriate technologies for the production of bioelectricity. The development of a bio dry cell is the right vision towards the realization of this idea. There is indeed the need to pursue this technology as the dependence on fossil fuels has caused more release of greenhouse gases into the atmosphere; leading to global environmental contamination is the primary cause of global warming. If the characteristics of the community rather than the individual microorganisms are of greater interest and potential application in future research, then the use of BDG for CLPP provides a relatively quick and straightforward way to obtain this information. Ecological stability and community response to disturbances are of particular interest in both natural and engineered environments such as the anodic chambers of MFCs, as these may be indications of overall system performance or potential failure. BDC microbial fuel cell is introduced to address systems where the community level problem is of more significant interest than that of individual microorganisms. The BDC microbial fuel cell provides a large ecological dataset based on the microbial metabolism of a mixed culture.

The dataset represents a series of responses based on the growth of microbes populations on 31 carbon sources contained in the Bio Dry Cell (BDC) chambers. The 31 carbon sources are the variables received in the BDC dataset. These variables may be interdependent because some of the carbon sources in the BDC are similar or metabolized in the same manner. This interdependency makes the dataset analysis particularly complicated and one solution to this analytical predicament is given which is based on the use of multivariate data analysis techniques.

Bio dry cell-based Microbial fuel cells (MFCs) are devices that use bacteria as the catalysts to oxidize organic and inorganic matter to generate electricity. Electrons formed by the bacteria from these substrates are conveyed to the anode mesh (negative terminal) and flow to the air cathode (positive terminal) linked by a conductive material comprising a resistor, or operated under a load (i.e., generating bioelectricity that runs a device). By convention, a positive current flow from the positive to the negative terminal, a direction opposite to that of electron flow. In most of the MFCs, the electrons that arrive at the air cathode merge among protons that diffuse from the anode through a membrane-less layer and oxygen presented from the air; the resulting product is water. In this study, the results of which continue described within, factors that influenced the generation of electric current is determined in a fabricated MFC, that uses glucose, banana peel, and pineapple peel as the substrate held examined and the most effective combination of values for the key factors. The fabricated MFCs of the system allows for air at the cathode part to enhance the degradation of solid waste while the anode part is anaerobic to maximize the generation of current.

It is the first-time banana peel, and pineapple peel are used respectively as substrates for MFC although cow manure held operated in another research.

The population growth is determined using the spectrophotometric method, and the microbial community in the anode part of the MFCs is distinguished biochemically and genetically, the effects of critical parameters like aeration and non-aeration moves are also examined.

This responsibility aims to find out how efficient the substrates understudy can be on the organisms of choice for the production of electricity by BDC-based microbial fuel cell technology. BDC-based MFCs are of interest as a technology for the conversion of carbohydrates to electricity. In particular reactors, bacteria transfer the electrons gained from their electron donor toward an anode. These electrons remain subsequently conducted over a resistance or power user toward a cathode, where oxygen or an alternative electron acceptor is reduced.

The implementation of an application system of offshore sediment-based electricity production toward small scale batteries and larger-scale bio-converters is done on a large scale. The output of an MFC is defined by the bacterial turnover rate, the internal resistance, and the activation overpotential at the electrodes. The bacterial turnover the standard is in support restricted by real parameters such as bacterial density, substrate adaptation, temperature, and pH.

The other two bottlenecks are from an electrochemical nature where the internal resistance of the microbial fuel cell is lowered by decreasing the distance between the anode and the air cathode electrodes and selecting the appropriate materials. The activation over potential signifies equipped by the electrode surface and the nature of the electron transport process from the bacteria to the electrodes.

The electron transfer is occurred either directly at the bacterial cell surface or by mobile, shuttling compounds that are produced by bacteria or added to the medium. The following addition is economically not viable in several cases. Additionally providing

catalysts within the electrode matrix may decrease the activation overpotential. As received a power density of 788 mW/m^2 by supplementing a stainless-steel mesh electrode with granular activated carbon, while no mediated plate allowed power densities of only $0.65mW/m^2$.

An aboveresultis obtained using a mixed culture; the power output results for axenic cultures are generally lower. They are using a combined culture fed with glucose obtained densities of up to $4310mW/m^2$ using an accommodated consortium. In this thesis work,the adapted consortium is capable of producing redox mediators in-situ; hence, the system mediates.One of the most promising reinforcements of MFCs is the energy-efficient treatment of wastewater, and organic waste treatment by conventional aeration now represents substantial energy expenditure. Alternatives are developed, such as up flowing anaerobic sludge blanket reactors. The technology enables energy recuperation by the digestion of organic matter toward biogas, which can hold appropriated into a combustion engine. However, the diluted characteristics of the domestic wastewater and the need for elevated temperatures hamper the direct anaerobic digestion of sewage. To mitigate the bottlenecks mentioned above and in particular to decrease the internal resistance, the MFC is redesigned that takes into account both the electrochemical and the biological requirements to obtain a higher power output.

Bio dry cell is a device that converts chemical energy into electrical energy using an enzyme as biocatalysts and organic waste as biofuels. Bio dry cells are particularly interesting as energy servers for portable electrical appliances. Therefore, bio dry cells, biofuel cells, and microbial fuel cells have many applications.

Bio dry cells' merits are more cheaply than other cells, and also they can operate on any condition, lower maintenance and operation cost. The MFCs and biofuel cells (BFCs) have still several issues to address beforementioned as low electrical performance and

moderate long-term stability. Nevertheless, MFCs and BFCs technologies are facing many challenges to be a reliable renewable energy source. Research in this field of the biological fuel cell must be continued to improve performances, nonetheless, from the previous studies.

It is evident that despite the prospect of organic waste as a captive energy resource in India and also inherent advantages of bio dry cell technology, no study has been made yet towards comprehensive performance evaluation of organic waste-based bio-dry cell electricity generation technology.

Thepresent work thus envisages the performative assessment of organic waste which supportsbio dry cell technology. Also, this research work confirms the improvement of bio dry cells with the conclusion of their advantageous and potential applications in future demand. Also, different key factors affecting bioelectricity generation on bio dry cells held examined, and these critical parameters remain present. Chapter 4 details the microbial ecology and operational performance of the two MFCs used throughout the second study in this research.

3.2 Materials and Experimental Methods

The materials and experimental methods stand separated into two sections. The MFC system is presented first followed by the Bio Dry cells, BDC innovative design, carbon source pulse tests (CSPTs), and naturally, the anaerobic procedure.The anaerobic sampling procedure is disclosed in detail in section 3.2.5. The BDC and data analysis procedures are presented in Section 3.3.

3.2.1 Microbial Fuel Cell System

The MFC system design and operation are essential to the overall experimental design, anaerobic sampling procedure, and associated ecological data. Chapter 2 presents a detailed account of the system design and maintenance.

3.2.2 Bio-Dry Cell

The photographs and schematics of the fabricated and fully assembled bio dry cell illustrated in Fig. 3.1.

(a)

(b)

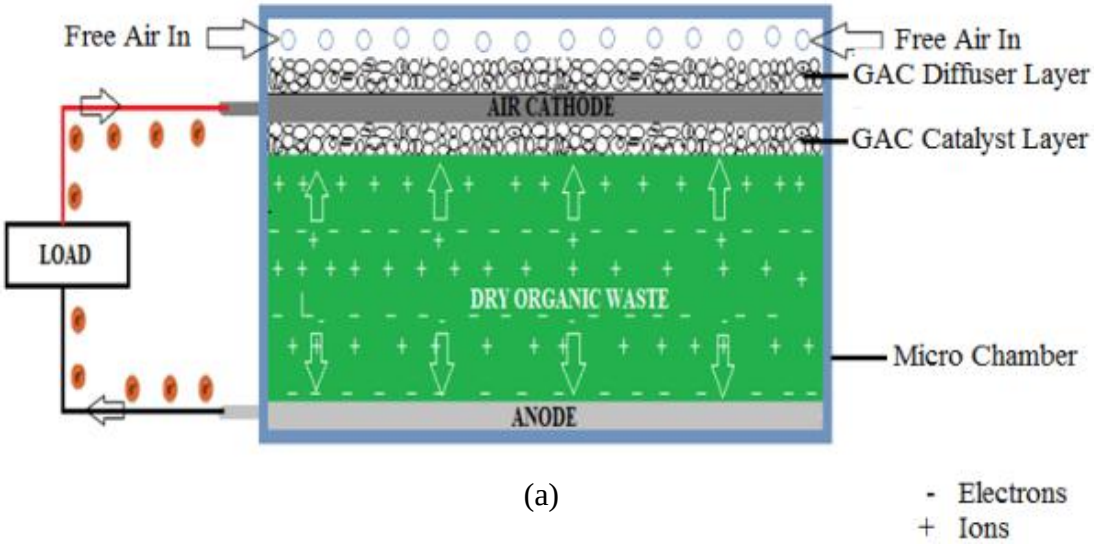
(c)

(d)

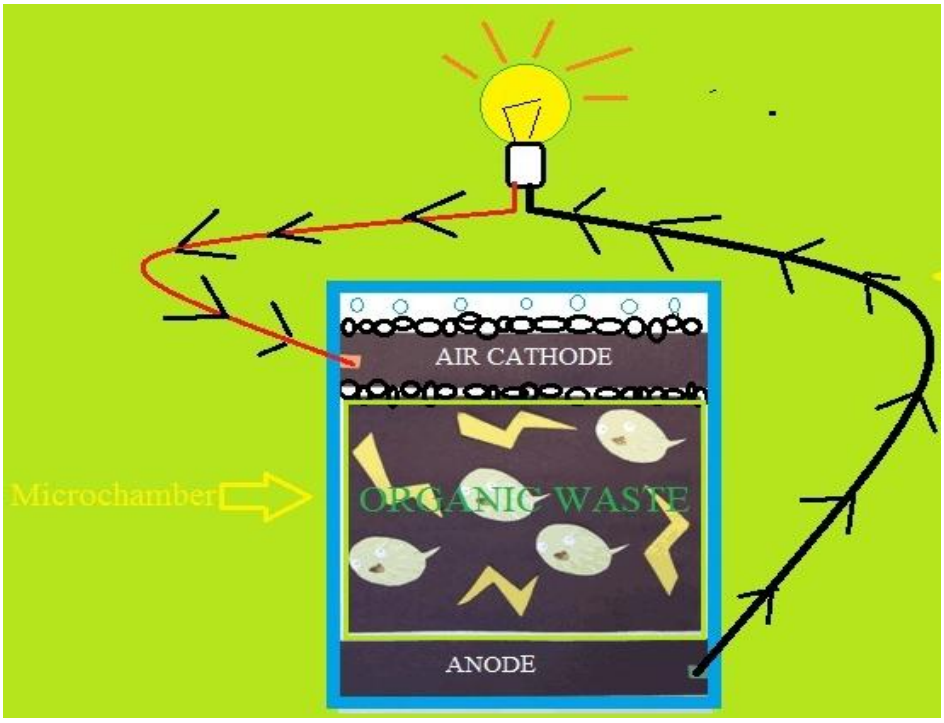
Figure 3.1: Schematic diagram of the fully assembled bio-dry cell device

The arrangement is enclosed by the brick-shaped microchamber, the indigenously designed bio dry cell consists of five different functional layers the top fifth layer is the granular activated carbon diffuser layer, and the fourth layer is air cathode comprising of

a stainless-steel mesh and below this, the third layer of granular activated carbon acts as a catalyst layer. The second layer consists of dry organic waste.



(a)



(b)

Figure 3.2 Principles of operation of bio dry cell

The last bottom layer consists of Aluminium mesh, which acts as an anode. All the individual layers are carefully aligned in a sandwiched configuration. Anode

aluminummesh and air cathode stainless steel mesh remain connected with two terminal blocks, which are plugged into the holes of the microchamber to form an electrical + ve and – ve output.The organic waste carbon sources as shown in Figure 3.2 are labeled as c0 through c31 for ease of reference and subsequent carbon source classification. From Figure 3.2, carbon source A1, which represents the empty well, is designated as c0 and labeling continued from left to right. Only the first four columns obtained specified since the last eight columns in the BDC are replicates for the carbon sources in the first four columns and have the same associated label. For example,

A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

Figure 3.3: Carbon Source Legend for the BDC(source)

Itaconic acid, shown as F3 in Figure 3.3, is labeled as c22. A classification system is developed to create a carbon source of organic waste from the kitchen of the domestic and agriculture-based biowastes of variable subsets for the purpose of further experimental setup PCA (Zak, Willig, Moorhead and Wildman, 1994). The carbon

sources are divided into four subgroups based on their general organic chemistry. The subsets are presented in Table 3.1 below:

Table 3.1: BDC Carbon Source Subset Classifications

Classification	**Carbon Sources**
Carbohydrates	c1,c5,c9,c13,c17,c24,c28
Carboxylic Acids	c2,c4,c6,c10,c14,c18,c21,c22,c26,c30
Polymers and Miscellaneous	c8,c12,c16,c20,c25,c29
Amines and Amino Acids	c3,c7,c11,c15,c19,c23,c27,c31

3.2.3 BDC Experimental Design

A total of seven BDC boxes are prepared throughout two experiments with the MFCs. During the first experiment, BDC boxes obtained only made from MFC#2, which is operated with a ferricyanide catholyte. The purpose of conducting the BDC boxes testing in the first experiment is to evaluate the difference between bulk anolyte samples and anode scraping samples. During the second experiment, BDC boxes are prepared from both of the MFCs.

The purpose of the BDC boxes testing in the second experiment was to evaluate the changes in microbial ecology overtime during system acclimation and after the carbon source pulse tests (CSPTs). Table 3.2 outlines the BDC boxes sampling timeline and experimental design significance. Table 3.3 presents the BDC boxes case organization for PCA.

A total of 4 sub-cases are accomplished for each of the cases in Table 3.3 based on the variable subsets presented in Table 3.1. All first sub-cases are based on the carbohydrate variable subset and held nominated as Case #x.1. Similarly, for the other

three variable subsets, sub-cases #x.2, #x.3 and #x.4 are designated for carboxylic acids, polymers/miscellaneous and amines/amino acids, respectively.

Table 3.2: BDC boxes Preparation Timeline

BDC boxes #	**Date Prepared**	**Design Significance**
Experiment#1		
1	October 17, 2016	1st Experiment Initial Feed
2	October 30, 2016	Centre point Bulk Solution
3	November 01, 2016	Centre point Anode Scraping
4	November 11, 2016	Endpoint Bulk Solution
5	November 13, 2016	Endpoint Anode Scraping
Experiment#2		
6	December 5, 2016	2nd Experiment Initial Feed
7	February 15, 2017	MFC#1 Steady State Operation
8	February 20, 2017	MFC#2 Steady State Operation
9	March 5, 2017	MFC#1 Post Sodium Acetate Dosing
10	March 7, 2017	MFC#2 Post Sodium Acetate Dosing
11	March 19, 2017	MFC#1 Post Glucose Dosing
12	March 21, 2017	MFC#2 Post Glucose Dosing
13	April 2, 2017	MFC#1 Post Glycerol Dosing
14	April 4, 2017	MFC#2 Post Glycerol Dosing
15	April 16, 2017	MFC#1 Post Bovine Serum Albumin Dosing
16	April 18, 2017	MFC#2 Post Bovine Serum Albumin Dosing

Table 3.3: BDC boxes Case Organization for PCA

Case #	BDC boxes used	Comparison of Significance
1.0	All	Entire ECO plate Set Comparison
2.0	2,3,4,5	Bulk Solution vs Anode Scraping Samples
3.0	1,4	Inoculant vs Endpoint (Experiment #1)
4.0	1,6	Inoculants Comparison
5.0	6,7,8	Inoculant vs Steady States (Experiment #2)
6.0	7,8,9,10	Pre and Post Sodium Acetate Comparison (both MFCs)
7.0	9,10,11,12	Pre and Post Glucose Comparison (both MFCs)
8.0	11,12,13,14	Pre and Post Glycerol Comparison (both MFCs)
9.0	13,14,15,16	Pre and Post BSA* Comparison (both MFCs)

*Bovine Serum Albumin

Table 3.4.: Carbon Source Pulse Test Substances

Substance	COD Mass per Pulse (mg)	Solution Preparation
Sodium Acetate	50 (1 pulse)	2.44137 g per 100 mL DI
	2.857 (3 pulses)	1 mL above solution to 16.5 mL DI
Glucose	2.857 (4 pulses)	0.08928 g per 100 mL DI
Glycerol	2.857 (4 pulses)	0.07831 g (0.0621 mL) per 100 mL DI
Bovine Serum Alumin (BSA)	2.857 (4 pulses)	0.06806 g per 100 mL DI

3.2.4 Carbon Source Pulse Tests (CSPTs)

The CSPTs involved the injection of a known chemical oxygen demand (COD) equivalence of a simplistic carbon source. The CSPTs took place after the MFCs had reached an operational steady state concerning current production. There are a total of 4 substances included in the CSPTs. Table 3.4 outlines these substances, in addition to the COD equivalent mass of each material, added, and solution preparation.

The choice of substances used in the CSPTs existed based on several factors. Uncomplicated contents are desired to minimize the microbial response time required before utilization could occur. Considering the anaerobic environment that is maintained in the anode chambers of each MFC, the substances are chosen based on their categorization in anaerobic digestion processes. Acetate as sodium acetate represented a low molecular weight volatile fatty acid, which is a typical intermediate in anaerobic digestion processes. Acetate is also appropriated by some methanogens to produce methane and carbon dioxide. Glucose represented a pure sugar or carbohydrate, while glycerol represented a lipid and bovine serum albumin (BSA) expressed a protein. In an anaerobic environment, each of these three carbon sources would continue biodegraded to simpler substances including organic acids, alcohols, ketones, carbon dioxide, and hydrogen. While bacteria likewise appropriate all three, they each represent a particular classification of substrate: carbohydrate, lipid, and protein.

The steps involved in the CSPTs are listed below:

1. Prepare each carbon source solution beforehand
2. Withdraw 3 mL from the prepared solutions with a syringe,
3. Inject carbon source solution into anode chamber via multi-functional, 1/2 inch NPT port in MFC lid, plug with rubber cap during MFC operation and throughout CSPT

4. The act of injecting the carbon source solution is called a `pulse'. Each carbon source is pulsed four times.

The pulses are spaced between sample/feed procedures, taking place every 48 hours. Once the fourth pulse is completed, the MFCs continued to operate for 2−4 days before samples arechosen for the ECOplate analyses. An ECOplate is prepared for each MFC, before and after the set of 4 pulses is completed for each carbon source.The first CSPT of sodium acetate had a COD equivalency of 50 mg COD. This value is chosen based on the soluble COD levels observed in the feed waste activated sludge. However, following this first pulse of sodium acetate, a period of higher current production is observed for both MFCs. The higher current levels are observed for almost four days. Subsequent CSPTs are performed with a COD equivalency of approximately 2.857 mg COD to avoid confounding electrical results from the CSPTs with regular sampling, and feeding showed every 48 hours. These levels of COD addition are less than 2% of the feed waste activated sludge COD addition levels. When considering the impact of the CSPTs on the CLPP and the microbial ecology as a whole, the deficient COD dosage levels suggest responses are more likely due to microbial activity shifts rather than a shift in the microbial population.

3.2.5 Anaerobic Sampling

As part of the experimental design, anaerobic conditions are maintained for the anolyte during sampling, BDC box inoculation, and analysis. If strict anaerobes comprised a significant portion of the viable and active biological community, oxygen exposure would be toxic and would have considerable effects on the microbial community.

The steps to the anaerobic sampling procedure are outlined below:

1. Gather equipment, BDC box of MFC and place in the glove bag
2. Establish an anaerobic environment in the glove bag using nitrogen gas

3. Collect an anolyte sample, dilute with buffer solution (4 parts buffer solution to1part anolyte sample) and manually homogenize with a Potter Elvehejm Homogenizer.
4. Measure the OD590 of the homogenized solution, repeat the previous step if the OD value is higher than 0.35 (dilution level may vary)
5. Oncethe homogenized solution has an OD reading of 0.25−0.35 at a wavelength of 590nm, plate out the resulting solution on the BDC box.
6. Seal the BDC box by placing the BDC box lid on the BDC box and taping the edge.
7. Open the glove bag to the atmosphere and immediately transfer the sealed BDC boxes to the microchamber reader for analysis

3.3 Analysis

3.3.1 Kinetic Optical Density (OD) Measurements

The description of the analytical procedures is divided into six sub-sections. The first section outlines the optical density (OD) measurement procedure, which is presented in greater detail in Appendix C. Once BDC box data are collected, the time point determination is carried out, followed by the data transformations. These transformations are evaluated against each other and the set of untransformed data based upon thestatistical constraints placed on PCA: normality, homoscedasticity and linear correlation of variables. The natural logarithm transform is found to be optimal, and PCA is performed on these transformed datasets to produce ordinate plots for the determination of CLPP.

From Table 3.3, only cases 1.0 to 4.0 with the associated sub-cases are presented and discussed in Section 3.4. Finally, BDC box data are used in a series of ecological tests to determine the functional diversity of the microbial community in the samples. The results of these analyses for BDC boxes 1 through 5 are presented and discussed in

Section.Once the BDC box is prepared anaerobically, the OD analysis is carried out. During BDC box analysis, the BDC box remained sealed with optical density (OD) readings taken through the microplate lid. A VERSA max tuneable microplate reader is used to make OD readings at 590nm every hour for 42 hours. The BDC box is incubated at 37 °C during OD measurements. The SOFTmax PRO 3.1.1 software allowed for automation of these readings and recording of the BDC box OD measurements for each time point into a textile. The maintenance of an anaerobic environment and continued incubation during analysis is a novel application of the BDC boxes.

The overall dataset consisted of 43 readings for each of 7 BDC boxes, each with 96 OD readings for the BDC box wells. This experiment resulted in over 66000 data points. The first step in data treatment is time point determination. The choice of a single time point for BDC box analysis and latter comparisons reduced the dataset by a factor of 43.

3.3.2 Time Point Determination

It is recommended by Garland (1996) that a single time point be used for useful comparisons between microplates (Weber, 2006). Also, the use of an individual, but optimal, time point reduces the analytical workload by a factor equal to the number of time points measured while preserving a maximum amount of the variance in the dataset.

The determination of the time point required preliminary numerical analysis. The standard deviation values of the 96 well OD readings at each time point for each BDC box are calculated. The number of well OD readings more significant than twois also calculated at each time point for each BDC box. These values are used to determine which time point or range of time points for each BDC box met the following criteria:

1. maximization of the variance between the 96 well OD readings measured at a time point
2. minimization of the number of well OD readings more significant than 2

The maximum OD reading of 2 represents the upper boundary of absorbance values that maintain a linear correlation with carbon source utilization in the BDC box chambers. Using these criteria, a range of optimal time points are identified for each BDC box. An overall optimal time point of 23 hours is chosen as the comparison point used in this study.

3.3.3 Data Transforms

Following the time point determination, data pre-treatment is performed. The data in each BDC box is normalized by the average well color development (AWCD) of the respective plate (Weber, 2006; Garland, 1996). Each OD measurement is also adjusted by the blank OD measurement, which represented a zero value on the OD measurement scale. Equation 3.1 illustrates this data

pre-treatment.

$$\overline{A}_K = \frac{A_K - A_0}{P_{i=1}(A_i - A_0)} \quad (3.1)$$

where,

$\overline{A}_K$ = pre-treated OD reading of well `k'

A_K: OD reading of well `k'

A_0: corresponding OD reading of the blank well on the BDC box containing well `k'

A_i: corresponding OD readings of the wells for each of the 31 carbon sources on the BDCbox containing well `k.'

This data pre-treatment helps to eliminate any biases of the original data due to excellent inoculation variability, allowing later PCA to be based on carbon source utilization differences. Another pre-treatment step recommended by Garland (1996) follows data normalization. All pre-treated OD values that are below zero are set to zero. Any well with a negative OD value relative to the blank of zero is considered non-

responsive, and equivalent to the blank. Negative OD readings can make PCA results challenging to interpret for CLPP. Similar to Weber et al. (2006), following data pre-treatment, the data are subjected to either no transformation, a Taylor power law transformation, or a natural logarithm transformation. These two transformations chosen by Weber et al.(2006) are common in the analysis of biological data and are considered equally valid for the MFC environmental data. The Taylor power law transformation is used to increase the homoscedasticity and the normality of a dataset (Legendre and Legendre, 1998). Equation 3.2 presents the fundamental assumption of the Taylor power law transformation. By taking the logarithm of each term in Equation 3.2, Equation 3.3 is derived.

A plot of the logarithm of carbon source variances versus the logarithm of carbon source means allows for the determination of the Taylor transform slope, `b'. The conviction of the Taylor transform slope is the required element for the application of the Taylor power law transformation. Equations 3.4 and 3.5 represent the transforms applied to the pre-treated data subject to a single constraint on the value Taylor transform slope.

$$S^2 = a.\overline{y.b} \tag{3.2}$$

where,

S^2= variance of a sample variable/carbon source

y=mean of a sample variable/carbon source

b=sampling factor

a=Taylor transform slope

Note: Each variation and mean value is obtained from data for a single carbon source across the BDC boxes applicable to the case being analyzed.

$$\log(S^2) = \log(a) + b.\log(\bar{y}) \tag{3.3}$$

where,

$$\overline{\mathrm{A}}_{\mathrm{K}} = A_K{}^{-(1-\frac{b}{2})} \quad (3.4)$$

$\overline{\mathrm{A}}_{\mathrm{K}}$: transformed and pre-treated OD reading of well `k.'

where,

$$\overline{\mathrm{A}}_{\mathrm{K}} = ln(A_K) \quad (3.5)$$

The natural logarithm transform is often used to normalize skewed datasets (Legendre andLegendre, 1998). Equation 3.6 represents the natural logarithm transform used in this study.

$$\overline{\mathrm{A}}_{\mathrm{K}} = ln(A_K + 1) \quad (3.6)$$

3.3.4. Statistical Constraint Diagnostics

The Taylor power law and natural logarithm transformed datasets are evaluated against each other and the non-transformed dataset. The datasets for each case defined in Table 3.3 are each assessed to ensure that the use of PCA applied to the datasets of each case. As discussed earlier, PCA is subject to several statistical constraints. The transformed and pre-treated datasets are evaluated based on the diagnostics of these mathematical constraints. The mathematical constraints are:

1. homogeneity of variance or homoscedasticity
2. normality of data
3. linear correlations between variables/carbon sources

Although it is difficult to evaluate the homogeneity of variance, the ratio of the highestvariance to the lowest variance is chosen for this study (Weber, 2006). Equation 3.7 illustrates the variance ratio as it is determined for each dataset.

The ratio is calculated by dividing the most considerable variance found for a carbon source in a dataset by the least variation found for a carbon source in the same dataset.

$$\text{Variance Ratio} = \frac{\text{Var high}}{\text{var low}} \quad (3.7)$$

where,Variance Ratio = ratio of most significant variable variance to least variable variation within a dataset Var_{high} = the most significant difference in a dataset, associated with one variable

Var_{low} = the least variance in a dataset, associated with one variable

The variance ratio approaches the desired value of one for complete variance homogeneity, but increases as the difference between the variances increases. However, there is a weakness in this evaluation when there is a consistent response in one variable since the variance is zero. The resulting variance ratio is always infinity regardless of how close to zero the most excellent variance value is. Also, variables with no response are not included in the PCA. To address the above issue, the definition of the least variance is adapted to:

Var_{low} = the least non-zero, variation in a dataset, associated with one variable. While this may appear to be an erroneous method to increase homoscedasticity, it should be recognized that the variable or carbon source with zero variance in the dataset is not included in subsequent PCA. A non-zero difference is required of every variable in PCA to complete the analysis and dene the principal components. Therefore, the removal of zero variances from datasets before homoscedasticity evaluation had no impact on the applicability of PCA to these datasets.

The normality of the pre-treated and transformed datasets in this study is evaluated using a series of statistical tests. The kurtosis of the datasets characterizes the peak of the

distribution relative to a normal distribution. Positive kurtosis indicates more peaks, while a negative value relates to a batter distribution. The skewness of the datasets characterizes the asymmetry of the distributions around their means. Positive skewness indicates an asymmetric tail in the distribution, extending toward positive values. Negative skewness indicates the same asymmetry in the distribution, but toward negative values. Kurtosis and skewness are calculated using predefined equations in Microsoft Excel.

The standard error for the kurtosis and skewness is needed to complete the diagnostics.Equations 3.8 and 3.9 represent the standard error for kurtosis and skewness, respectively.

$$SE_{Kurtosis} = r\frac{\overline{24}}{n} \tag{3.8}$$

Where,

$SE_{Kurtosis}$ = the standard error of the kurtosis

n = the number of data points within a dataset or data subset

$$SE_{Skewness} = r\frac{\overline{6}}{n} \tag{3.9}$$

where,

$SE_{Skewness}$ = the standard error of the skewness

Statistical methods for the diagnostic tests require a z-value to determine normality at a 95% confidence level. Z-values for the kurtosis and skewness of the datasets are calculated from Equations 3.10 and 3.11.

$$Z_{Kurtosis} = \frac{Kurtosis}{SE_{Kurtosis}} \tag{3.10}$$

where,

$Z_{Kurtosis}$ = the z-value of the kurtosis

kurtosis = the kurtosis of the dataset

$$Z_{Skewness} = \frac{Skewness}{SE_{Skewness}} \quad (3.11)$$

where,

$Z_{Skewness}$ = the z-value of the skewness

Skewness = the skewness of the dataset

The data are considered generally distributed if the absolute values of the z kurtosis and $Z_{skewness}$are less than 1.96 for a 95% confidence level. Optimally conditioned datasets would contain most of the z-values close to or equal to zero. Two methods are used to determine the normality of the datasets, following the procedure of Weber et al., (2006). In the first method, the averages of the absolute values for the kurtosis and skewness of each of the 31 variables or carbon sources are used to determine a $Z_{kurtosis}$ and $Z_{skewness}$. In the second method, the kurtosis and skewness values for all of the 31 variables or carbon sources are used to calculate a $Z_{kurtosis}$ and $Z_{skewness}$for each of the 31 variables.

All of these z-values are evaluated at the 95% confidence level, and the number of significantly typical z-values for each full dataset is compared. Finally, the correlation between variables isassessed for linearity using a correlation matrix for the 31 variables or carbon sources. If the absolute value of the correlation coefficient ismore significant than Pearson's critical r-value, the two variables are considered linearly correlated with a 95% confidence level. Pearson's critical r-valuesare chosen based on the number of observations or objects that constitute the dataset. The total number of linearly correlated variables is calculated, and this value is used as a comparative basis between the pre-treated and transformed data, where more linearly correlated variables arefavored.

3.3.5 Principal Component Analysis (PCA)

PCA is a multivariate analysis technique based on the analysis of an R-mode, variance-covariance matrix. PCA is used with datasets in higher dimensional spaces by

coordinating objects from the dataset on a 2-dimensional plane while preserving the maximum amount of variance contained in the dataset.

This leads to the determination of any shifts or differences between samples or objects based on the number of variables (Legendre, 1998) within this study, the carbon sources are the variables, constituting a 31-dimensional space. The objects for PCA are the BDC box well replicate sets. Therefore, each BDC box consisted of three objects, since there are triplicate sets of carbon source on each BDC box. The result of performing PCA is the ordination of the dataset objects on a 2-dimensional space for visual analysis.

The first two principal components, or eigenvectors, are used throughout the PCA of the datasets in this study. As mentioned earlier, this minimizes the impact of having fewer objects than variables, which is true for most of the analyzed cases. Typically, 40% to 80% of the original dataset variance is contained within the first two principal component axes. Based on these properties and the recommendations of Garland (1996), PCA is used to analyze the BDC box datasets from this study.

3.3.6 Functional Diversity

The data from the BDC box are also subjected to another analysis to evaluate the functional diversity of the samples, as suggested by Zak et al. (1994). Weber et al. (2006) carried out a similar analysis using the BDC box as compared to the BIOLOG ®gram negative and gram-positive microchamber used by Zak et al. (1994). Zak et al. (1994) also defined functional diversity as the numbers, types, activities, and rates at which a suite of substrates are utilized by the bacterial community. The BDC box preparation timeline presented earlier in Table 3.2 provided the samples for the functional diversity analysis. Each BDC box is evaluated for the useful diversity indices: substrate diversity, substrate richness, and substrate evenness.

The term `substrate diversity' is used because the BDC box data represented substrate utilization patterns rather than microbial colony direct counts from inoculated nutrient agar plates. Substrate diversity (H) is calculated from Equation 3.12, as derived by Weber et al.(2006):

$$H = \frac{X}{P_i \ln(P_i)} \tag{3.12}$$

where,

P_i = ratio of the pre-treated OD reading of a particular substrate to the sum of the pre-treated OD readings of all substrates.

The substrate richness (S) equals the number of different substrates utilized by the microbial community within the sample. As there are a maximum of 31 carbon source substrates in the BDC box, the substrate richness values for this study are integers bounded by 0 and 31 A substrate is considered utilized if the pre-treated optical density value is greater than 0.25.

The substrate evenness is defined as the equitability of activities across all utilized substrates. Substrate evenness (E) is calculated from Equation 3.13, as derived by Weber et al. (2006):

$$E = \frac{H}{\log(S)} \tag{3.13}$$

The inherent triplicates in the pre-treated BDC box data areanalyzed using the above equations, and average substrate diversity, evenness, and richness are calculated for each BDC box. The substrate diversity, abundance, and evenness are normalized to the first BDC box of each experiment to allow for normalized comparison. Therefore, BDC boxes 1 through 5 are normalized to BD box 1, while BDC boxes 7 through 16 are normalized to BDC box 7.

3.4 Results and Discussion

The presentation and discussion of results are separated into three sub-sections. The pre-treated and transformed datasets are compared and evaluated using the statistical constraint diagnostics outlined in Section 3.3.4. The natural logarithm transformed dataset is found to be optimal. PCA is performed on this dataset using the cases summarized in Table 3.3 and the sub-cases discussed in Section 3.2.3. Finally, functional diversity is evaluated for each BDC box.

3.5 Conclusion

This study revealed that the microbial fuel cells based Bio Dry Cell fabricated have a great ability to be used to house the substrates and organisms in an anaerobic environment for electricity generation. Fermentation of the three different substrates such as glucose, banana peel and pineapple peel for energy production is successfully carried out in the single chamber of the fabricated MFCs. The interesting point is that sources of electron donors have a significant role in the efficiency of the microbial fuel cells. Results obtained showed that the seven BDC boxes for MFC successfully produced bioelectricity from different kinds of organic wastes without using any mediator in the anode compartments. The maximum current and power obtained for the effect of aeration/non-aeration are 1.17 X 10-3 A, 8.99 X 10-4W and 998mA, respectively. The electricity supply in the H shape microbial fuel cell is unstable. Therefore, more research is needed to combat the internal resistance and produce better electrodes and devices that will trap the electricity for significant scale purposes.