STATISTICAL EVALUATION FOR BACTERIA ELECTRO-STIMULATION USING THE DUNNETT METHOD FOR A MICROBIAL FUEL CELL

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Received 31.07.2023. DOI: 10.29039/rusjbpc.2023.0646

Abstract. A microbial fuel cell is a bioelectrochemical device that uses microorganisms, such as electrogenic bacteria, capable of generating electricity. However, the electrical energy generated depends mainly on the ability of the microorganisms present in the anode to decompose the organic matter contained in an effluent. In this work, an electrochemical setting formed as an anode and a cathode of graphite felt were connected in an electrical circuit to electro-stimulate microorganisms to enhance the biofilm on the anode. In this sense, different potential values were imposed in several electrochemical cells to evaluate chemical oxygen demand, volatile solids, and bacteria increase. Dunnett method was used to find significant differences between treatments, taking a sample without treatment as the control sample.

Key words: *Electrogenic bacteria, biofilm, Dunnett method, Saphiro-Wilk, Variance analysis.*

INTRODUCTION

The first discoveries in the use of microorganisms to obtain electric energy were made in 1911 by Potter, who developed the first prototype of a microbial fuel cell (MFC) by experimenting with *Escherichia coli* and Saccharomyces cultures, as well as the use of platinum electrodes, with which he obtained a small magnitude of electric currents in a range of 0.3 to 0.5 V [1-3].

MFC is considered an environmental alternative to efficiently and comprehensively solve the problems related to sustainably obtaining electricity. This is possible because the electrical energy generated in a CCM depends primarily on the capacity of the microorganisms present in the anode to decompose the organic matter contained in an effluent [4].

Biofilm formation occurs after the establishment of inoculum bacteria on the electrode surface, which occurs by the action of Van der Waals forces, acid-base interactions, and electrostatic forces [5].

Biofilm formation by bacterial clustering has demonstrated electroactivity and susceptibility to electrical stimulation, which is why electrochemical techniques expand the options for biofilm characterization and modification [5].

METHODOLOGY

In order to analyze the comparative effects obtained by pretreatment of the anode electrode, graphite-felt electrodes were used. The anode pretreatment consisted of modifying the electrode surface to improve compatibility with the electrogenic bacteria present in the inoculum and thus promote biofilm formation on the anode. Two pretreatments were applied on graphite felt, and the untreated graphite felt was used as a control. In that sense, three graphite-felt samples were obtained named: ST (untreated), TT (thermal treatment), and ET (electrochemical treatment).

The electrochemical treatment (ET) was based on the methodology of Cercado 2013 [6]. The geometric area treated was 2 x 2 x 0.3 cm, using a potentiostat-galvanostat (VoltaLab PGZ 301) to apply 1.6 V vs. Ag as a pseudo reference for one hour. The phosphate buffer solution was used as an electrolyte media (4.33 g/L Na_2HPO_4, 2.69 g/L NaH_2PO_4, 2.85 g/L NaCl and 1.0 g/L CH_3COONa).

The thermal treatment (TT) consisted of calcinating at 400 °C the graphite felt into a muffle (Thermolyne, Thermo Scientific) for 20 minutes and then cooling at room temperature.

Once the anodes were pre-treated, they were placed in the fuel cell, according to Figure 1. Different electrical cell potential values were applied for 110 hours using a power source (GEINSTEK GPE-2323 and GP-1303DU E2 Digital Co.). In this case, 0 V, 0,5 V, 1.1 V, and 2.2 V were applied. At the end of this time, the percentage of organic matter was evaluated as the total suspended solids (TSS) and chemical oxygen demand (COD). However, both analyses were compared with a control system (a cell without applying the electrical potential).

Comparison of treatments concerning the control (Dunnett method). Let **k** treatments be compared, one of which will be called the control treatment. The main interest is to compare the remaining (k – 1) treatments with the control treatment. In many cases, the control treatment refers to a reference standard treatment or the absence of treatment. The (k-th) treatment is denoted as the control treatment.

Making comparisons concerning the control involves testing the (k – 1) hypotheses given by:

$$H_0: \mu_i = \mu_k$$
$$H_A: \mu_i \neq \mu_k \quad (1)$$

With i= 1, 2, …, k – 1, where k is the control treatment. The null hypothesis is rejected if:

$$|\bar{Y}_{i.} - \bar{Y}_{k.}| > D_\alpha(k-1, l) = \sqrt{CM_E\left(\frac{1}{n_i} + \frac{1}{n_k}\right)} \quad (2)$$

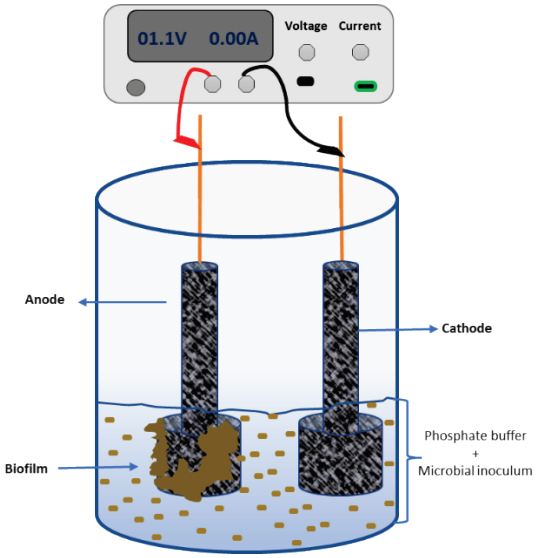

Figure 1. Electrochemical cell used for biofilm formation

Where D_α (k-1, l) is the following: D by Dunnet, α the level of significance, and Dα is found in Dunnet's tables; l are the degrees of freedom.

$\bar{Y}_{i.}$ y $\bar{Y}_{k.}$: They are the sample means of the treatments.

CM_E : Is the mean square of the error.

n_i: Is the number of elements contained in each treatment.

n_k: Is the number of elements contained in the control treatment.

An Excel spreadsheet was used to carry out this test, as shown in Figure 2. This program was applied to obtain the corresponding results [7-10].

RESULTS AND DISCUSSION

The data obtained from the experiment carried out are shown in Table 1, considering the methods Without Treatment, Thermal Treatment, and Electrochemical Treatment, applying potentials from 0.5 V, 1.1 V, 2.2 V, and 0 V for the sample Without Treatment.

Statistical analysis was carried out on the data in Table 1, which consisted of applying the Shapiro-Wilk normality test, and the Levene Test to determine equality of variances, which are the necessary requirements to apply an Analysis of the Variance of two Factors, with a single sample per group. If this analysis of variance shows significant differences, then Dunnett Test can be applied.

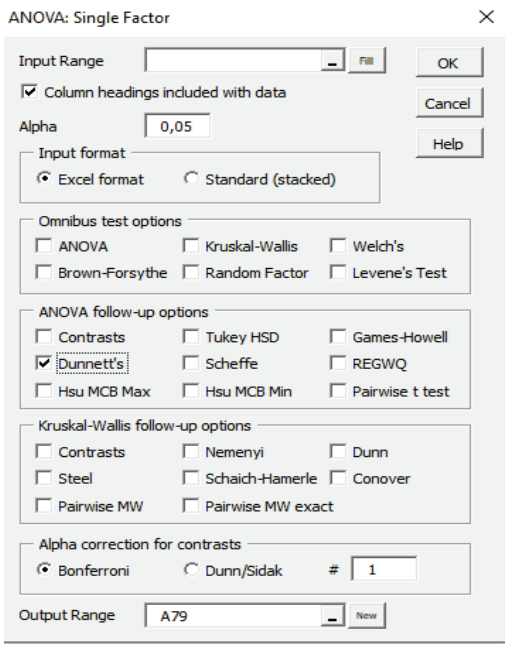

Figure 2. Dunnett test calculation with an Excel program

What is attempted to be demonstrated through the Dunnett test is whether there are significant differences in the treatments to conclude which treatment worked better. Dunnett method allows to compare the treatments with a control treatment, determined by the investigator, which for our analysis was the sample Without Treatment.

To apply the Dunnett test, we will first use the normality test to the data in Table 1, then the variance equality test, all this before applying an Analysis of Variance.

The first thing we must corroborate is the normality of the data. This will determine what statistical analysis to perform, whether parametric methods because they have a normal distribution or non-parametric methods if they are not normal, for which we apply the Shapiro-Wilk test using the Excel spreadsheet, which showed the following results. See Table 2.

As seen in Table 2, the Shapiro-Wilk test demonstrated normality in the data, so we proceeded to determine equality of variances, to cover the requirement needed to apply an Analysis of Variance.

To verify the equality of variances, the Levene test was applied using the Excel spreadsheet, and the results shown in Table 3 were obtained.

As can be seen in Table 3, the test shows that the p-value is greater than 0.05, indicating that the variances do not have significant differences, so equality of variances is assumed. Since the requirements for applying two-factor Analysis of Variance were met, we proceeded again to use the Excel spreadsheet. The results obtained are shown in Table 4.

As can be seen in Table 4, if F is less than the critical F 2.24132898 < 5.14325285, it is concluded that there are no significant differences concerning the methods. On the contrary, if there are significant differences where F is greater than the critical F 8.14827786 > 4.757062663 (Table 4) it can be concluded that there are statistically significant

Table 1. Data showing the percentage of organic matter with different cell potentials

	Treatments			
Methods	0 Volts	0 .5 Volts	1 .1 Volts	2 .2 Volts
ST	44.19	44.19	50.91	33.07
TT	43.48	37.50	45.45	29.51
TE	43.75	53.68	46.34	35.48

Table 2. Data showing the percentage of organic matter at different cell potentials

Shapiro-Wilk Test	***0 Volts***	***0.5 Volts***	***1.1 Volts***	***2.2 Volts***
W-stat	0.98235356	0.99003646	0.86821869	0 .9878123
p-value	0.7455413	0.80904353	0.29047929	0.78872389
alpha	0.05	0.05	0.05	0.05
normal	yes	yes	yes	yes

Table 3. Levene test to determine equality of variances

Levene's Tests	
type	p-value
means	0.095471186
medians	0.240998795
trimmed	0.095471186

Table 4. Analysis of variance of two factors with a single sample per group

Analysis of variance of two factors with a single sample per group,						
Origin of variations	*Sum of squares*	Degrees of freedom	Average of the squares	**F**	Probability	Critical Value for F
Methods	71.73252867	2	35.86626434	2.24132898	0.18751651	5.14325285
Treatments	391.167076	3	130.3890253	8.14817786	0.01545379	4.757062663
Error	96.01338669	6	16.00223112			
Total	558.9129913	11				

differences, but the Analysis of Variance does not indicate in which treatment these differences occur. However, if there were differences, Dunnett Test can be applied using the sample called without treatment as a control test to compare it with the other treatments and determine where there are differences. The results are shown in Table 5.

Table 5 shows that it is in the treatment of 2,2 V, where a significant difference is shown with respect to the control treatment. It can be commented that if the p-value is very close to 1, there are no differences in these treatments, but if this value is close to zero or zero, there are differences.

In order to verify the differences in the treatments, we will show other results obtained, applying the method of mean differences, and the results are shown below. See Table 6.

It can be seen in table 6 that if we compare the control treatment called 0 Volts with the 2,2 Volts treatment, the result shows that if there are significant differences, this result coincides with that obtained by applying Dunnett's Test.

CONCLUSIONS

When an experiment is carried out, where it is tried to demonstrate the efficacy of one treatment with respect to another, it is important to know if the data provided by the experiment show significant differences for our investigation, the treatments used to determine the percentage of organic matter, there were 3 methods and 4 treatments; the methods are Without Treatment, Thermal Treatment and Electrochemical and the treatments were with voltage 0, 0.5, 1.1, and 2.2 volts.

In the results obtained, we were able to observe that when comparing the control treatment with the 2,2 treatment, it was evidenced that there are significant differences with respect to the control treatment, as shown in Tables 5 and 6, when applying the Dunnett test and that of Differences of Means.

In conclusion, the Dunnett test indicated that there are significant differences in the analysis process of a water sample with different treatments, which showed us that in the treatment with voltage 2.2 V there was a statistically significant difference with respect to the sample without treatment, a situation which was corroborated with the physical

Table 5. Dunnett test

DUNNETT'S TEST			alpha	0,05				
group	*mean*	*size*	*ss*	*df*	*d-crit*			
0 volts	43.80	3	0.25497974					
0.5 volts	45.12	3	132.282336					
1.1 volts	47.57	3	17.1339702					
2.2 volts	32.69	3	18.0746295					
		12	167.745915	8	2.88			
D-TEST								
group	*mean*	*std err*	*d-stat*	*lower*	*upper*	*p-value*	*mean-crit*	*Cohen d*
0.5 volts	-1.32	3.73882686	0.35269081	-12.0864712	9.44917147	1	10.7678214	0.28797084
1.1 volts	-3.76	3.73882686	1.00662524	-14.5314188	7.00422389	1	10.7678214	0.82190606
2.2 volts	11.12	3.73882686	2.97342587	0.34930316	21.8849459	**0.04372891**	10.7678214	2.42779206

Table 6. Test of differences of means

CONTRAST			Alpha	0,05
Group	*contrast*	*mean*	*size*	*ss*
0 volts	1	43.80	3	0.25497974
0.5 volts		45.12	3	132.282336
1.1 volts		47.57	3	17.1339702
2.2 volts	-1	32.69	3	18.0746295
	0	11.117124	12	167.745915

T TEST									
std err	*t-stat*	*df*	*p-value*	*t-crit*	*lower*	*upper*	*sig*	*Cohen d*	*effect r*
3.738826859	2.97342587	8	0.01777791	2.30600414	2.49537432	19.7388747	**yes**	2.42779206	0.72455241

experiment carried out. The electrochemical treatment method with voltage 2.2 V is the one that showed the lowest percentage of organic matter, as can be verified in Table 1. And we could say that it was the best treatment.

For future work, 3 repetitions will be applied for each treatment, in order to determine, with more information, if the electrochemical process with voltage 2.2 is the one with the lowest percentage of organic matter, with respect to the other treatments.

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ИСПОЛЬЗОВАНИЕМ МЕТОДА ДАННЕТА ДЛЯ МИКРОБНОГО ТОПЛИВНОГО ЭЛЕМЕНТА

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Поступила в редакцию 31.07.2023. DOI: 10.29039/rusjbpc.2023.0646

Аннотация. Микробный топливный элемент – это биоэлектрохимическое устройство, в котором используются микроорганизмы, такие как электрогенные бактерии, способные вырабатывать электричество. Однако вырабатываемая электрическая энергия зависит главным образом от способности микроорганизмов, присутствующих на аноде, разлагать органические вещества, содержащиеся в сточных водах. В данной работе электрохимическая установка, выполненная в виде анода и катода из графитового войлока, была соединена в электрическую цепь для электростимуляции микроорганизмов для усиления биопленки на аноде. В этом смысле в нескольких электрохимических ячейках были установлены разные значения потенциала для оценки химической потребности в кислороде, летучих твердых веществ и увеличения количества бактерий. Метод Даннетта использовался для обнаружения существенных различий между обработками, принимая образец без лечения в качестве контрольного образца.

Ключевые слова: *Электрогенные бактерии, биопленка, метод Даннета, Сафиро-Уилка, дисперсионный анализ.*