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STUDY EXPERIENCE OF USING PLANT MATERIALS INFECTED BY FUNGI AS SUBSTRATE FOR MICROBIAL FUEL CELL

A.I. KUCHEROV, O.Y. NECHYPORUK, K.O. PALIIENKO,
H.K. SAVCHENKO, L.S. TYMOSHENKO, Y.V. YUZVENKO,
A.V. DRAZHNIKOVA
National Aviation University, Kyiv

Article presents the results of experimental investigation applicability of plant fallen materials contaminated by powdery mildew fungus *E. flexuosa* as substrate in microbial fuel cell (MFC). MCFs were presented in form of Winogradsky's column. The measurements of generated voltage were carried out in MFCs with different composition during 5 weeks.

Key words: Winogradsky column, electrogenesis, microbial fuel cell, fungi, substrate, plant material.

Introduction. One of a main source of phytopathogenic fungi in urban environment is decaying plant material of infected decorative trees. Infected fallen leaves must be utilized in order to prevent distribution of fungi. That is why such plant material can be used only as substrate in production of bioenergy. One of such technology is based on the usage of infected plant materials as substrate for microbial fuel cell (MFC).

We choose Winogradsky column as type of microbial fuel cell. It consists of such elements as:

- cellulose or plant leaves as a source of carbon;
- lake mud as a source of electrogenic microbial community;
- sulphates as a source of sulfur.

Due to different concentration of oxygen and sulfur at the top and at the bottom of column, that creates condition for growth of different groups of bacteria, the excess of electrons is generated at the bottom.

Winogradsky columns are successfully used as MFC for effective removal of color from azo dye mixtures and to simultaneously generate bio-electricity [1], for biohydrogen production [2] and for evaluation of ecotoxicity [3].

Purpose of work is to evaluate the effect of plant contamination by fungi on the production of bioelectricity in MFC. Proposed null hypothesis: there isn't any influence of fungal contamination of substrate on microbial electrogenesis.

Materials and methods. Fig. 1 shows a block-scheme of the research.

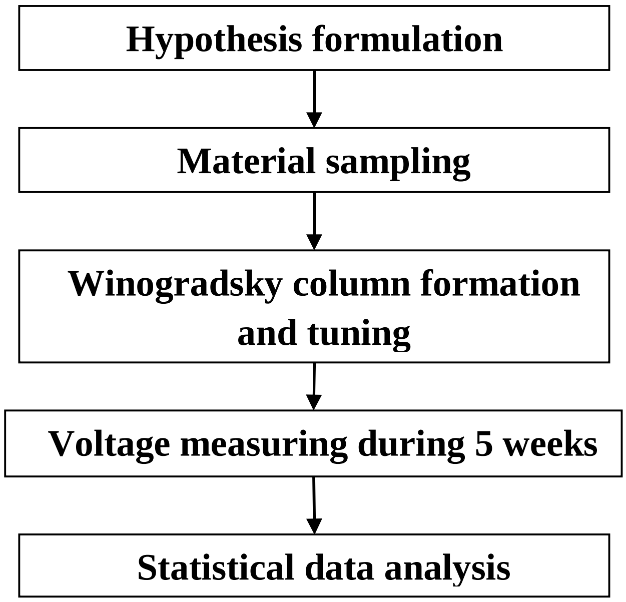

Fig. 1. Scheme of research

To confirm or reject a formulated null hypothesis it was set up Winogradsky columns with different component content of the carbon source.

In September of 2016 year on the territory of National Aviation University fallen leaves of horse chestnut (*Aesculus hippocastanum* L.) were collected, including leaves infected by powdery mildew fungus (*Erysiphe flexuosa* (Peck) U. Braun & S. Takamatsu) as well as mud and water from lake Integral in Vidradny park. Collected leaves from chestnut trees were sorted «infected» and «uninfected» by presence or absence of *E. flexuosa* cleistothecia on the bottom surface of leaves that were identified with help of defectoscope МПБ-3 (fig. 2).

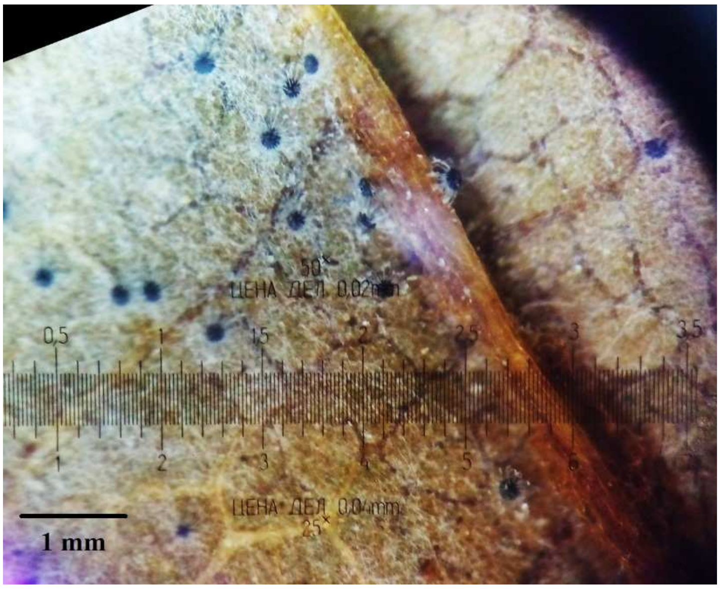

Fig. 2. *E. flexuosa* cleistothecia on the bottom surface of leaves

Winogradsky columns were set up in a 1500 ml plastic bottles according to the general procedure described in “Benson: Microbiological Applications Lab Manual” [4]. They were be filled with mud, sulfate, lake water and a source of fermentable cellulose and protein. The cellulose, in this experiment, was be in the form of dead plant leaves infected by fungi.

The total composition of Winogradsky column is presented in table 1.

Table 1

Composition of Winogradsky column

Chestnut pericarp, g	Seed of chestnut, g	Carbon source, g	Mud, ml	$MgSO_4 \cdot 2H_2O$, g
100	50	25	30	1

Three types of columns were made with different cellulose source: infected leaves, uninfected leaves, filter paper as control. Each type of column was established with 3 replicates.

Seeds of chestnut where used as source of protein. According to Cukanovic J. et al. [5] it has 5.96 % protein. Lake mud was used as a source of association of electrogenic microorganisms. $MgSO_4 \cdot 2H_2O$ was used as a source of sulfur for enrichment of bacteria that are involved in the sulfur cycle.

Columns incubation was performed for 5 weeks. At first week columns were stored in dark place at room temperature for prevention of ameba growth. At second week columns were stored in thermostat at temperature 37 °C for intensification of microbial cellulose decaying processes. Next 3 weeks columns were incubated at room temperature and exposed to incandescent light for enhancement growth of autotrophic microorganisms.

Each week voltage in column was measured. For measuring of voltage contact-making millivoltmeter (60 mV) was used. As conductor of electrical current were used copper wire with 50 cm length and 3 mm in diameter.

Statistical analysis of obtained data was carried out in program MS Excel with the help of ANOVA test. Conclusions about rejection or acceptance of null hypothesis were made according to the results of the test.

Results and discussions. Obtained experimental data from determination of microbial electrogenesis intensity in MFC with different component composition are presented in table 2.

Table 2

Intensity of microbial electrogenesis in different MFC, mV

13.10.2016	Batch	1	2	3	Mean	Variance
	Cellulose	4,3	3,8	6,07	4,72	1,42
	Infected	2,53	2,8	2,4	2,58	0,04
	Uninfected	2,3	1,9	1,4	1,87	0,2
20.10.2016	Batch	1	2	3	Mean	Variance
	Cellulose	2,00	2,70	2,73	2,48	0,17
	Infected	1,60	1,43	1,77	1,60	0,03
	Uninfected	1,43	1,33	1,67	1,48	0,03
27.10.2016	Batch	1	2	3	Mean	Variance
	Cellulose	0,9	0,97	0,9	0,92	0,001
	Infected	0,53	0,90	0,93	0,79	0,05
	Uninfected	0,77	0,73	0,80	0,77	0,001
03.11.2016	Batch	1	2	3	Mean	Variance
	Cellulose	0,9	0,73	0,7	0,78	0,01
	Infected	0,63	0,73	0,8	0,72	0,01
	Uninfected	0,67	0,97	0,73	0,79	0,02
10.11.2016	Batch	1	2	3	Mean	Variance
	Cellulose	0,5	0,9	0,8	0,73	0,04
	Infected	0,7	0,47	0,7	0,62	0,02
	Uninfected	0,73	0,77	0,7	0,73	0,001

Next stage was statistical analysis of obtained data with help of ANOVA test. Results are presented in table 3.

Table 3

ANOVA test data

Parameters				Between groups			Within groups			Total	
Week	*F*	F_{crit}	*P-value*	*SS*	*df*	*MS*	*SS*	*df*	*MS*	*SS*	*df*
1	11.963		0.008	13.251		6.625	3.323		0.554	16.573	
2	11.720		0.008	1.785		0.893	0.457		0.076	2.242	
3	1.229	5.143	0.357	0.042	2	0.021	0.104	6	0.017	0.146	8
4	0.265		0.776	0.008		0.004	0.087		0.014	0.094	
5	0.592		0.583	0.025		0.012	0.125		0.021	0.150	

Ratio between *F* and F_{crit} give us possibility to make a conclusion about null hypothesis. According to data that were obtained due to measuring of voltage in columns in first 2 weeks of incubation null hypothesis was rejected. It was determined significant difference in average microbial electrogenesis between the three batches: columns with infected leaves, uninfected leaves and control ones. In next 3 weeks hypothesis was accepted. Analysis of data obtained in next three weeks of incubation indicates the absence of significant difference in average electrogenesis between the three batches.

It was approved that infected leaves could be used as substrate for microbial fuel cell. Sharp decline of voltage for first 2 weeks of incubation can be explained by assumption, that some amount of electrons were present from the beginning and oxidative reactions which proceeded gradually used it:

$$O_2^0 + 4\bar{e} = 2O_2^{2-}$$

CONCLUSION

For utilization of fallen leaves contaminated by powdery mildew fungus *E. flexuosa* it was proposed as substrate in microbial fuel cell. We determined absence of influence of fungal contamination on electrogenes efficiency in MFC, developed as Winogradsky column, since 3 week of incubation.

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ДОСВІД ВИКОРИСТАННЯ ІНФІКОВАНОЇ ГРИБАМИ РОСЛИННОЇ СИРОВИНИ ЯК СУБСТРАТУ ДЛЯ МІКРОБНОГО ПАЛИВНОГО ЕЛЕМЕНТУ

А.І. КУЧЕРОВ, О.Ю. НЕЧИПОРУК, К.О. ПАЛІЄНКО, Г.К. САВЧЕРНКО, Л.С. ТИМОШЕНКО, Ю.В. ЮЗВЕНКО, А.В. ДРАЖНІКОВА

Національний авіаційний університет, м. Київ

У статті наведено результати експериментального дослідження можливості застосування контамінованого збудником борошнистої роси *E. flexuosa* листового опаду каштана в якості субстрату для мікробного паливного елементу (МПЕ). МПЕ розробляли за принципом колонки Виноградського. Проводили вимірювання напруги упродовж 5 тижнів інкубування МПЕ різного компонентного складу.

Ключові слова: колонка Виноградського, електрогенез, мікробний паливний елемент, гриби, субстрат, рослинна сировина.

ОПЫТ ИСПОЛЬЗОВАНИЯ ИНФИЦИРОВАННОГО ГРИБАМИ РАСТИТЕЛЬНОГО СЫРЬЯ В КАЧЕСТВЕ СУБСТРАТА ДЛЯ МИКРОБНОГО ТОПЛИВНОГО ЭЛЕМЕНТА

А.И. КУЧЕРОВ, Е.Ю. НЕЧИПОРУК, К.О. ПАЛИЕНКО, А.К. САВЧЕНКО, Л.С. ТИМОШЕНКО, Ю.В. ЮЗВЕНКО, А.В. ДРАЖНИКОВА

Национальный авиационный университет, г. Киев

В статье представлены результаты экспериментального исследования возможности применения контаминированного возбудителем мучнистой росы *E. flexuosa* листовой опад каштана в качестве субстрата для микробного топливного элемента (МТЭ). МТЭ разрабатывали по принципу колонки Виноградского. Проводили измерения напряжения в течение 5 недель инкубации МТЭ различного компонентного состава.

Ключевые слова: колонка Виноградского, электрогенез, микробный топливный элемент, грибы, субстрат, растительное сырье.