IIETA International Information and Engineering Technology Association
Advancing the World of Information and Engineering

Journal of New Materials for Electrochemical Systems
Vol. 27, No. 3, September 2024, pp. 182-189
Journal homepage: http://iieta.org/journals/jnmes

Development of Polypyrrole/Cellulose Composite based Pseudocapacitive Bioelectrode for Enhanced performance of Biofuel Cell

Aynul Rifaya. M[1], Venkatesa Prabhu Sundramurthy[2,3*], Shine Kadaikunnan[4], and Jamal M. Khaled[4]

[1]Department of Chemical Engineering, Érode Sengunthar Engineering College, Érode, India
[2]Department of Biotechnology, Faculty of Engineering, Karpagam Academy of Higher Education, Coimbatore 641 021, Tamil Nadu, India
[3]Centre for Natural Products and Functional Foods, Karpagam Academy of Higher Education, Coimbatore 641 021, Tamil Nadu, India
[4]Department of Botany and Microbiology, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia

Corresponding Author Email:haiitsvp@gmail.com

https://doi.org/10.14447/jnmes.v27i3.a03

Received: 20/01/2024
Accepted: 31/08/2024

Keywords:
biofuel cell, multi-walled carbon nanotubes (MWCNT), polypyrrole, Fructose dehydrogenase (FDH), supercapacitor

ABSTRACT

Optimized enzymatic electrodes utilizing a polypyrrole/cellulose composite with high internal capacitance were developed for an enhanced enzymatic fuel cell. Gluconobacter sp. fructose dehydrogenase enzyme was electrophoretically bound and fixed to the polypyrrole/cellulose matrix. Laccase from Trametes versicolor was utilized as the cathode bioelectrocatalyst, being entrapped electrochemically. The composite film made of polypyrrole and cellulose displayed pseudocapacitive characteristics in neutral pH circumstances. After being modified with carboxylic groups, the composite material facilitated effective enzyme adsorption as well as ensured proper electrical connection between the enzyme's active sites as well as the electrode exterior. The updated polypyrrole/cellulose composite electrode was employed as the anode for fructose oxidation without a mediator, achieving a significant catalytic current density of 13.1mA cm^{-2}. Dioxygen decreases at 3 mA/cm^2 current density was achieved by a composite film that contained laccase and was made of polypyrrole and cellulose. The film contained 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) as a mediator. Utilizing a pseudocapacitive matrix as well as reducing the electrode distance to 1.5 mm enhanced the power output as well as regeneration capacity of the biofuel cell. Implementing a preconditioning stage where the cell was held at open circuit voltage while fuel flows has been found to improve the cell's power output. After the matrix was recharged after a 24-h preconditioning time, the device produced 3.1 mW/cm2 power and an open-circuit voltage of 0.61 V.

1. INTRODUCTION

Interest in energy collecting systems using alternative fuels has greatly raised during the last twenty years[1]. Electrochemists are currently showing a particular interest in biofuel cells due to the limitations of existing fuel cell technologies despite nearing mass manufacturing. The growing interest in biofuel cells is due to their distinctive operational requirements that are not achievable by traditional fuel cells, such as operating at low temperatures (20 – 40ºC), functioning near neutral pH levels, the enzymes' specificity, selectivity as well as the reducing cost of biocatalysts. Furthermore, biofuel cells can use a variety of fuels as well as different biocatalysts, while also providing cost-effective operation and maintenance compared to traditional fuel cells [2], [3], [4], [5].

Despite notable advancements in this subject in recent years, there are still substantial challenges that need to be addressed. A common issue is the quick decline in voltage that frequently occurs while activating biofuel cell-operated devices. Electric capacitance-based techniques are being studied in contemporary literature because to their association with power limits of systems[6]. These technologies are crucial for delivering or storing high power quickly. Furthermore, storing the electric energy generated by the biofuel cell involves complex processes such as electrostatically or by electrochemical reactions, adding to the efficiency challenges. Traditional capacitors and batteries, frequently utilized for storage are inefficient as well as costly devices plagued by fundamental and practical limitations including self-discharge.

Supercapacitors (SCs) [7], batteries[8]as well as their combinations are rechargeable devices capable of storing larger amounts of electric energy compared to traditional electronics[9]. Energy storage systems, such cutting-edge fuel cells, are being constructed using sophisticated nanomaterials mostly made of carbon, such as carbon nanotubes (CNTs) as well as graphene[9]. Carbon-based supercapacitors are considered crucial energy repository tools because to their outstanding characteristics, such as high-power densities, adequate electrical conductivity, cost-effectiveness, and inertness. Additionally, organic polymers, such as polypyrrole

(PPy)[10] and polyaniline (PANI)[11] are very hopeful and commonly utilized electrode materials in supercapacitors due to their accessibility as well as high particular capacitance, reaching up to around 3500 F g^{-1}[12]. Batteries and supercapacitors are unable to generate electrical energy independently and must be charged outside. The enzymatic fuel cell (EFC) transforms chemical energy into electrical energy by utilizing nanostructured electrodes composed of carbon as well as metals like CNTs and gold nanoparticles. The electrodes are utilized to create extremely effective EFCs, similar to advanced electrochemical capacitors[13]. Although enhanced nano-biostructures are utilized, the current as well as power densities of biodevices remain notably lower than those of fuel cells available on the market. To improve the EFCs' efficiency, external capacitors are used [14].

It is worth noting that many hybrid system configurations involving fuel cells and capacitors have been documented in the literature elsewhere. These combinations include EFCs with conventional capacitors, EFC/SC hybrids and conventional FCs with a SC[15].It is significant to note that prior articles examined a unique device consisting of two separate modules: a fuel cell as well as a capacitor, which were coupled electrically and assessed. Pankratov and colleagues demonstrated the initial work involving a single module that serves as both a supercapacitor as well as a fuel cell being one and invisible[16], [17]. In a flash, the researchers tested a biological catalyst-based enzymatic self-recharging SC[18]. On each side of the electrodes in the biodevice are components that charge or act as pseudocapacitors. Agnes and her coworkers showcased the refilling capability of a capacitor-like enzymatic fuel cell with the use of carbon nanotubes[19]. The first single electrode surface with an enzyme that generates current and a matrix that collects charges was recently demonstrated in our work[20], [21].

In this study, the production process of bioelectrodes using a pseudocapacitive polypyrrole/cellulose matrix was demonstrated in order toenhance the biofuel cell's efficiency that can be achieved by including carboxylic groups into the polypyrrole/cellulose composite for facilitating better and directed fructose dehydrogenase adsorption [22]. For this purpose. electrophoresis was used to immobilize the laccase enzyme on the matrix structure. Then, to make the cathode more durable, electropolymerized polypyrrole was used as a coating[23].Further, the correlation between the hybrid material's internal capacitance and the BFC's output power was explained.

2.EXPERIMENTAL METHODOLOGY

2.1 Materials

Fructose ($C_6H_{12}O_6$),citric acid ($C_6H_8O_7$), ethanol (C_2H_5OH) as well as disodium hydrogen phosphate (Na_2HPO_4), and multi-walled carbon nanotubes were acquired from Sigma-Aldrich, Mumbai, India. The multi-walled carbon nanotubes were modified with naphthalene groups in accordance with an earlier study[24]. Using a Milli-Q purification system, the water was distilled followed by filtered and utilized.

Laccase *Trametes versicolor* (activity ≥ 12U/mg) and FDH from *Gluconobacter sp* were procured from Sigma Aldrich and Karpagam Academy of Higher Education, respectively. Extra purification was not performed on the enzymes before use.

The following materials were used as unmodified: Pyrrole-2-carboxylic acid ($C_5H_5NO_2$) (SigmaAldrich), Pyrrole (Merck), NaCl (Sigma Aldrich), $FeCl_3\cdot 6\ H_2O$ (Sigma Aldrich) 37% HCl (Merck) and Tween-80 (Merck).In order to reach the necessary amounts when mixed with deionized water. As described by Yang et al., the cellulose that was extracted from the algae *Cladophora sp*. and utilized [25].

2.2 Preparation and analysis of materials

The process for preparing the cellulose/polypyrrolecomposite (CPPy) have been adopted from the reports documented by Hui et al. and Wu et al.[26], [27]. The composite exterior was functionalized with carboxylic groups by dispersing 90 mg of CPPy in 10 ml of 0.5 HCl as well as sonicating it for 80 min to achieve a stable and uniform solution. After that, combine 15 milliliters of 0.5 M HCl with 0.01 M $C_5H_5 NO_2$ and 0.01 M $FeCl_3$.For 45 minutes while stirring, the polymerization process was carried out. After collecting the final item using a Buchner funnel, it was washed with solutions of 1.0 M HCl as well as 0.2 M NaCl.

Agate mortar was used to mix the composite (cellulose/polypyrrole composite) or the -COOH altered composite (cellulose/polypyrrole composite -COOH) with acetylene black in a 90:10 ratio, creating the bioanode of the biofuel cell.A concentration of 20 mg ml^{-1} was achieved by dispersing the mixture in 96% ethanol using high-energy ultrasonication. After that, a glassy carbon electrode (GCE, BAS) with an outside area of 0.071 cm^2 was coated with 80 µl of the solution. There was a 1.0 mm thickness to the layer. Afterwards, the GCE surface was treated with 60 µl of an FDH solution containing 25 mg ml^{-1} of enzyme, and the electrodes were placed in the refrigerator for the night to let the solvent drain.

A CPPy cathode with laccase adsorbed on its composite surface (cellulose/polypyrrole composite - Lac) as well as another with immobilized laccase in conjunction with the mediator ABTS-Lac were both investigated. An overnight immersion in a laccase solution (35 mg in 1 ml of a 1:1 C_2H_6O-buffer mixture of McIlvaine) was followed by the application of 25 µl of cellulose/polypyrrole composite suspension onto the glassy carbon electrode surface to create the CPPy-Lac electrode. An electropolymerized polypyrrole film was used to encapsulate the enzyme and mediator, resulting in the system (cellulose/polypyrrole composite - ABTS - Lac). To create the cellulose/polypyrrole composite -ABTS-Lac electrode, the cellulose/polypyrrole composite -altered GCE was mixed with 50 mg ml^{-1} of laccase, pyrrole (15 mM), as well as ABTS (15 mM). To improve the enzyme dispersion in the polymeric composites, the electrode was pulsed with 4 V for 10 seconds and then 0.5 V for 3 seconds, a total of 170 times. To remove any molecules that were loosely adhered to the electrode, deionized water was used to wash it thoroughly.

Naphtylated MWCNT were used to fabricate the –nanostructured electrode surface. The process of chemically altering carbon nanotubes by attaching naphthyl groups has been explained in a different source [28]. Eight milligrams of Napht-CNTs were dispersed in twelve milliliters of C_2H_6O as well as subjected to sonication for thirty minutes. Later, 50 µl of multi-walled carbon nanotube suspension was applied onto the glassy carbon electrode surface and let to dry. For enzyme adsorption, the MWCNT electrode was immersed in a 35 mg ml^{-1} laccase solution and let to sit in the fridge overnight.

The electrochemical tests performed in an indigenous chamber. A plexiglass tube with two GCE, each with a diameter of 0.9 cm and positioned opposite each other, makes up this stationary, unsealed system. An optional Ag/AgCl reference electrode can also be inserted into its designated slot. You may fine-tune the distance between the electrodes using the see-through tube. A three-electrode setup with a programmable decade resistor was used to monitor the biofuel cell's power. Because of this, the biofuel operation may control the voltage of each electrode individually. Quantification of the voltage across the anode as well as cathode was done with loads ranging between 0.1 and 10 MΩ. A steady output voltage was guaranteed by capturing each data point in the power graph 10 seconds after adding each resistance upload [30-32].

Cyclic voltammograms (CV) were taken in a three-electrode setup, consisting of the bioelectrode, a reference electrode made of Silver/Silver chloride (KCl sat.), and a working electrode made of platinum foil to evaluate the bioelectrodes' catalytic capabilities.

3.FINDINGS AND ANALYSIS

3.1Attributes of the anode coated with (CPPy) and fructose

Fig. 1a shows a capacitive-shaped voltammogram obtained using the CPPy – FDH electrode without fructose present. While experimentation, 50 µl of a 2 M fructose solution was added, the chromatoamperogram for fructose oxidation in a pH 5.3 McIlvaine buffer at a constant potential of +0.4 V vs Ag/AgCl for the CPPy - FDH (blue line) and CPPy - COOH - FDH (green line) electrodes, respectively. Above roughly +0.4, the enhance in the anodic current was due to the beginning of polypyrrole overoxidation. As illustrated by the dashed line, catalytic waves were not visible in the absenteeism of Fructose dehydrogenase.

At approximately -0.15 V relative to Ag/AgCl, a catalytic wave of fructose oxidation started after fructose was added. The electron transmission occurs by the direct electron transfer (DET) process, as previously explained [22]. At approximately +0.15 V, the wave leveled off, perhaps because of kinetic constraints. The most recent data shows that the highest current density for the fructose solution at a concentration of 0.15 M is 9.1±1.2 mA cm^{-2}.

Figure 1 (B and C) shows that the cellulose/polypyrrole composite - COOH - FDH electrode exhibited comparable catalytic characteristics, with a catalytic potential of -0.16 V against Silver/Silver chloride. Within the specified potential range, the voltammogram retains its capacitive characteristics even in the absence of the catalytic reaction substrate. The catalytic current stabilized at a voltage of +0.36 V and a current density of 15.2±1.6 mA/cm2, in contrast to the cellulose/polypyrrole composite - fructose dehydrogenase electrode (when compared to Ag/AgCl). Current density increases, suggesting that the carboxylic group on the matrix enhances the electrode's catalytic capabilities for fructose oxidation (Fig. 1 B).

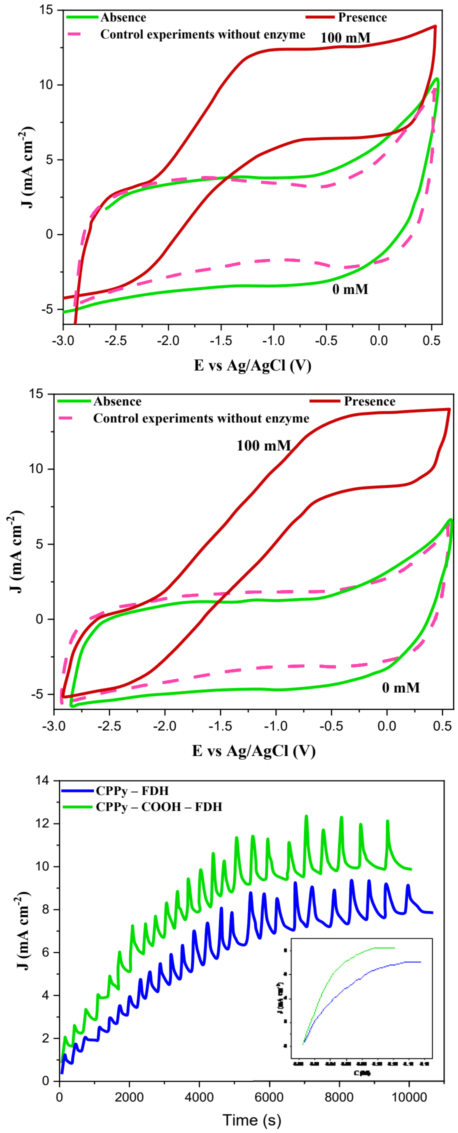


Figure 1. (A) An electrode consisting of Cellulose polypyrrole and fructose dehydrogenase, and (B) an electrode consisting of CPPy and COOH and FDH, were both measured in a pH 5.3 McIlvaine buffer with a scanning rate of 1 mV/s. In the presence (blue line) and absence (green line) of 100 mM fructose, the experiments were carried out as follows: (C) control experiments without enzyme in the presence of 100 mM fructose, shown by the blue dashed lines

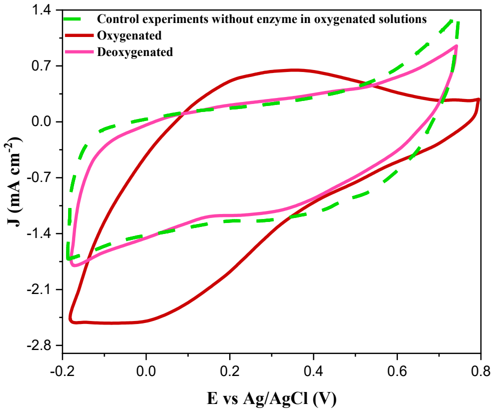
Chronoamperometry was used to determine the specific properties of fructose dehydrogenase systems. An electrode was subjected to a possible of +0.5 V versus Silver/Silver chloride in the limiting current zone and left at this potential for around 2 hours to reach equilibrium. After the background current had stabilized, 50 µl of a 2 M fructose solution was injected, and the catalytic current was measured many times (Fig. 1 C).

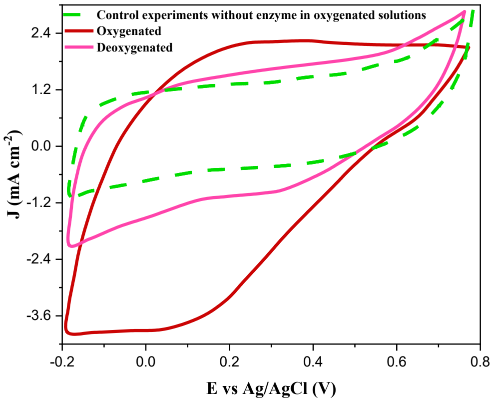
With each addition of fructose solution, the catalytic current rose for both types of electrodes. The CPPy – FDH electrode had a linear range of 0to 25 mM, whereas the cellulose/polypyrrole composite – COOH – FDH electrode had a range of 0 to 38 mM. Both electrodes had Michaelis-Menten constants (KM) of 26.8 mM and 45 mM, respectively. Consistent with the results of the cyclic voltammetry studies, the highest catalytic present densities attained were 9.68 mA/cm^2 for cellulose/polypyrrole composite - FDH as well as14.13 mA/cm^2 for cellulose/polypyrrole composite - COOH - FDH.

3.2 Characteristics of the cellulose/polypyrrole composite and Laccase cathodes

Cyclic voltammetry was used to study the performances of the cathodes based on CPPy. Figure 2A shows the voltammogram of the CPPy – Lac electrode with and without oxygen. Without oxygen, the anodic peak around +0.5 V as well as the cathodic peak around +0.1 V vs Silver/Silver chloride represent the oxidation as well as decrease of polypyrrole, respectively. Chronoamperogram was conducted for oxygen reduction in pH 6.2 McIlvaine buffer at a potential of 0 V vs Ag/AgCl while adding 2.5 µl of oxygen saturated solution. The inset shows the catalytic current density plotted against the oxygen concentration

The higher background current induced by CPPy probably made it impossible to differentiate the redox peak of the enzyme's active site in an oxygen-free environment. No catalytic waves were seen when laccase was not present. A catalytic reduction wave appeared at around +0.5 V compared to Silver/Silver chloride in an oxygen-saturated solution. Because the T1 copper site in Trametes versicolor laccase has an official potential of 0.78 V vs Silver/Silver chloride, electron transport is associated with an overpotential in the absence of a mediator. At approximately 0 V, the wave current achieves a stable plateau. As a result of polypyrrole oxidation, a current of about +0.3 V relative to silver/silver chloride is probably present. A current of 1.2±0.1 mA cm^{-2} was the highest recorded during the process of reducing oxygen.





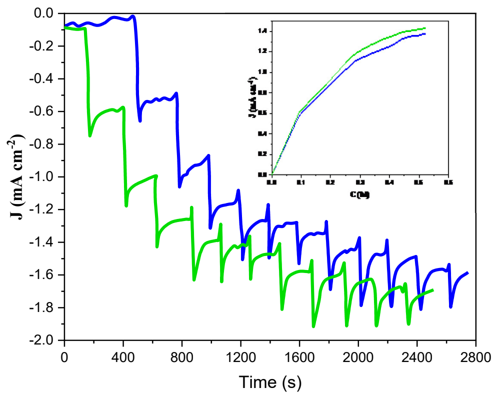


Figure 2. Cyclic voltammograms were obtained in pH 5.3 McIlvaine buffer at a scan rate of 1 mV s-^{1} for CPPy – Lac and CPPy – ABTS – Lac electrodes. The studies were carried out in a deoxygenated (green line) and oxygenated (blue line) solution. The green dashed line represents control experiments conducted without enzyme in oxygenated solutions

The electrode holding the mediator, CPPy – ABTS – Lac, functioned as the cathode for oxygen decrease, with catalytic current initiating at around +0.5 V against Ag/AgCl. Overpotential was decreased by the ABTS mediator in the matrix, leading to improved electron exchange between the electrode surface as well as the active region of the enzyme. In this case, as shown in Figure 2B, the maximal catalytic current density was 3.2±0.42 mA/cm^2, and the catalytic wave reached a plateau near 0 V in comparison to Silver/Silver chloride.

The chronoamperometric study indicated a potential within the limiting current zone by using an electrode voltage of 0 V relative to Silver/Silver chloride. Following approximately two hours of keeping the electrode at this voltage, the catalytic currents were monitored following the addition of 2.5 µl of an oxygen-saturated buffer sample, as shown in Fig. 2C. Whether electron transfer was mediated or direct, the catalytic current increased as oxygen-saturated solutions were applied to both electrodes. Calibration curves as well as Lineweaver-Burk curves were created to characterize the kinetics of the processes. At an oxygen concentration near 0.6 mM, both cases showed peak current on the curves, suggesting that variations in current density could be due to varying enzyme concentrations. The CPPy – Lac as well as the CPPy – ABTS - Lac electrodes have a linear range from 0 mM to 23 mM. The values obtained from the CV results were in good agreement with the maximal current densities of catalysis for the cellulose/polypyrrole composite - Lac electrode and the cellulose/polypyrrole composite - ABTS - Lac electrode, which were 2.5 mA/cm^2 and 3.0 mA/cm^2, respectively. The ABTS mediator elevates the reduction onset potential while maintaining nearly constant catalytic current.

3.3 Assessment of biofuel cell characteristics

In order to ascertain the electrode-derived fuel cell properties and the impact of the biofuel cell's internal capacitance on power generation, we investigated three systems. A symmetrical fuel cell uses a composite of cellulose for both electrodes. Three different electrodes are used in this setup: one is based on the cellulose/polypyrrole composite -COOH-FDH / cellulose/polypyrrole composite -ABTS-Lac formation, one is a hybrid cell with a cellulose/polypyrrole composite - Fructose dehydrogenase anode and a glassy carbon electrode cathode coated with naphthylated Multi-walled carbon nanotubes and Lac, and the third is a cellulose/polypyrrole composite -FDH / cellulose/polypyrrole composite -Lac formation.

The experimental approach called for measuring the current after 10 seconds and using resistance-reducing loads in the biofuel cell circuit.A hybrid fuel cell containing naphthylated multi-walled carbon nanotubes as well as adsorbed laccase electrodes demonstrated an enhanced open circuit voltage (OCV) of +0.81 V, as shown in Figure 3A. However, it also showed a faster response when the upload resistance was reduced compared to the symmetric CPPy-founded systems. Lower OCV values (0.62 V and 0.75 V, respectively) were observed in the CPPy-based systems. The highest power density of 3.0 mW/cm^2 was achieved by combining CPPy with FDH and Lac. Figure 3B illustrates the relationship between voltage and current in the circuit. An initial voltage drop due to kinetic limitations is observed in all circumstances. In the center of the graphs, you can see a plateau for all three cells; however, the combination of cellulose/polypyrrole composite - COOH - FDH / cellulose/polypyrrole composite - ABTS - Lac had the most noticeable impact. At large present densities, the voltage loss is due to mass transport, specifically diffusion constraints.

Figure 3 shows that the open-circuit voltage (OCV) of the system with the MWCNT-founded cathode (0.82 V) was significantly higher than that of the CPPy electrode, which were 0.73 V as well as 0.69 V vs Silver/Silver chloride. When the current density was first raised, the voltage of the multi-walled carbon nanotubes -based cell decreased more rapidly. At high current densities, the voltage drop was less pronounced in the cellulose/polypyrrole composite - COOH - FDH / cellulose/polypyrrole composite - ABTS - Lac arrangement compared to the symmetric EFCs, which showed a similar increase with rising current density.

Several possible modifications in the biofuel cell's operating parameters should be brought about by the differences in maximum current densities between the anode and cathode catalytic currents, as shown by the cyclic voltammetry observations. The symmetrical curves in symmetrical systems show that the electrodes are more stable during the experiment because they have a high and similar internal capacitance.

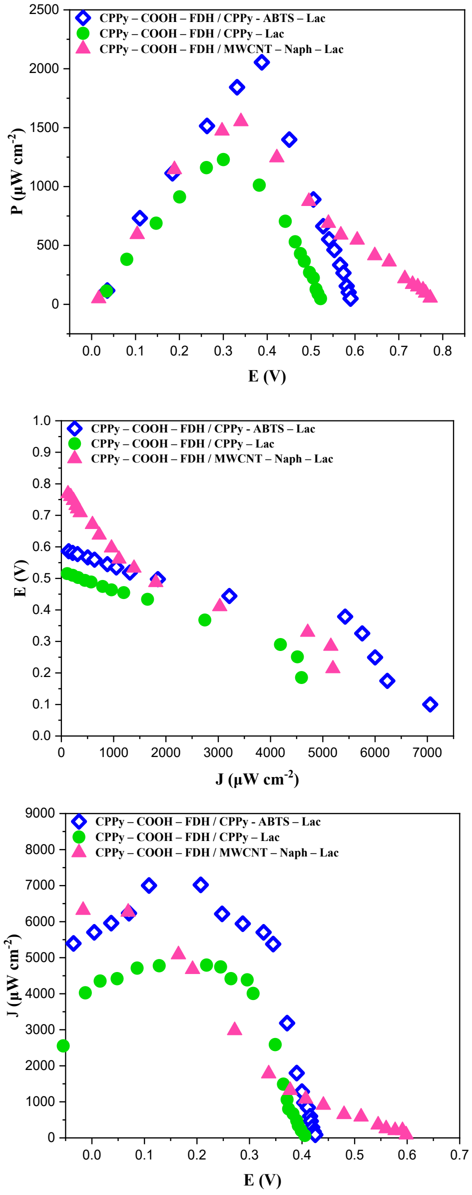


Figure 3. (A)Power is a function of the fuel cell voltage,(B) voltage is a function of current density, (C) generated current is a function of the voltage of each fuel cell electrode (anode Va and cathode Vc) for various cell configurations

A symmetrical system consisting of CPPy, COOH, FDH, ABTS, and Lac showed power output that varied with time. Both electrodes' potentials and power output increased after conditioning in a fuel solution for 24 hours before starting the experiment (refer to Fig. 4A). This action is brought about by enzymes that, under certain circumstances, produce current at an open circuit voltage, which continuously charges the pseudocapacitive polypyrrole/cellulose matrix. This is the reason for the increase in potential of the electrodes over time. Figure 4B shows that after charging the polypyrrole matrix, the enzymatic fuel cell reached its maximum OCV. The electrode potential decrease grew more symmetrical over time, which can be attributed to the current charging effect as seen in Figure 5A. The impact of electrode distance in the fuel cell was assessed by varying the distances between electrodes to 0.1cm, 0.6cm, 0.9cm, 2cm and 3 cm. The various configurations of biofuel cells are illustrated by their power profiles in Figure 5. The arrangement with the shortest electrode distance of 0.15 cm demonstrated reduced voltage drops, leading to an induced voltage of 0.4 V as well as an effective power output about twice as high at 3.5±0.2 mW as the arrangement with the longest electrode distance of 4 cm, which produced a power output of 2±0.21 mW at 0.35 V. The electrodes of conventional supercapacitors are typically separated by a thin, permeable membrane that is excellent at conducting ions and measures less than 1 mm in thickness [29]. Two symmetric enzymatic systems were evaluated for stability over a one-week period, with the normalized power as well as OCV values for each day displayed in Figure 6. Equation (1) was used to obtain the power value by integrating the observed curve I versus E:

$$P=E\int I dt \qquad (1)$$

The system that included the imprisoned enzyme and ABTS became more stable as time went on. Despite a forty-percent drop in final power production, the fuel cell's open-circuit voltage remained stable for the whole week. One week after being loaded with CPPY-FDH / CPPy-Lactase enzymes, the symmetrical fuel cell's voltage plummeted to 0.45 V, holding onto only 9% of its original power. Both the device's stability and its power output are dictated by the cathode.

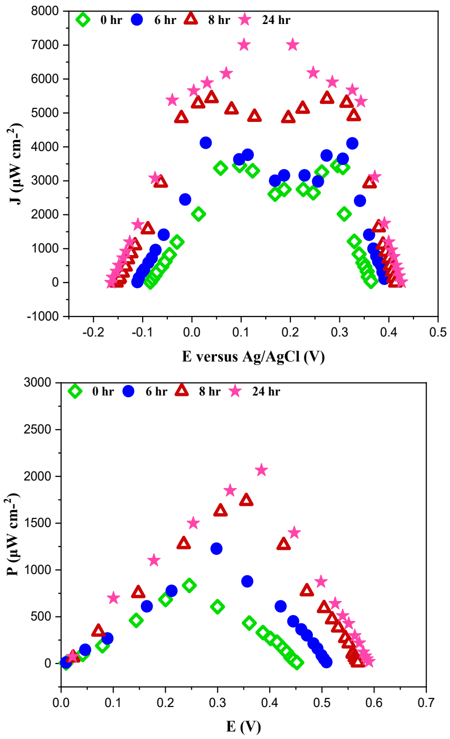


Figure 4. (A)The produced current dependent on the potential of each fuel cell electrode: anode (Va) and cathode (Vc). (B)The generated power related to the cell voltage for different conditioning times for the CPPy – COOH – FDH / CPPy - ABTS – Lac configuration: 24 h, 8 h, 6 h, 0 h

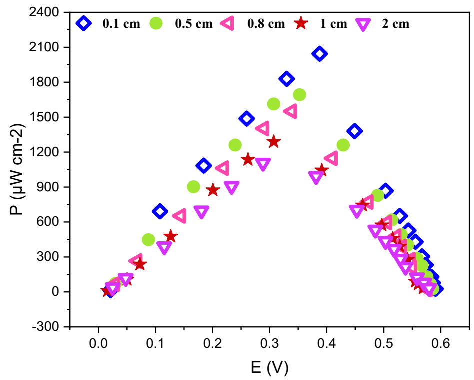


Figure 5. Power is plotted as a function of cell voltage for the CPPy – COOH – FDH / CPPy - ABTS – Lac system at various distances between the electrodes after 24 hours of conditioning: 0.1 cm; 0.5 cm; 0.8 cm; 1 cm; 2 cm.

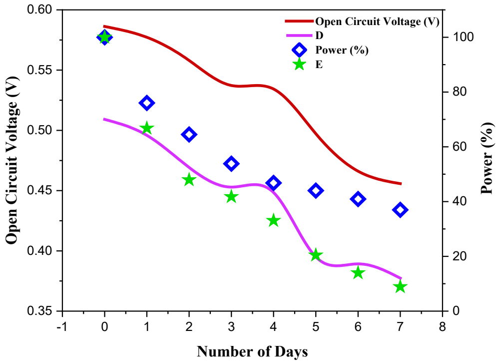


Figure 6. Relationship between normalized power and open circuit voltage over time for different enzyme combinations.

4. CONCLUSION

The anode and cathode of a biofuel cell were modified utilizing FDH and laccase, respectively, and the electrodes were pseudocapacitive polypyrrole/cellulose composites. The addition of a carboxylic group to the composite increased the catalytic current density to 16 mA/cm^2, made the electrode more durable, as well as allowed for more FDH adsorption. In contrast to Silver/Silver chloride, the composite electrode's potential increased to +0.45 V due to the presence of ABTS as a mediator and the adsorption of laccase. The electrode was able to sustain its catalytic activity for seven days and achieve a peak present density of 3.2 mA/cm^2.

The biofuel cell tested under a 1 kΩ load showed an open-circuit voltage of 0.7 V, a peak current density of 8.9 mA/cm^2, and a maximum power density of 3.5 mW/cm^2, all thanks to the bioelectrodes that made it up. Although the cathode and anode displayed almost symmetrical current against electrode potential curves, the cyclic voltammetry data indicated that the max catalytic currents generated were various for each electrode. Capacitance current is an essential component of the overall electrical output. The power density was discovered to be influenced by both the charging time as well as the distance separating the electrodes. The biofuel cell's OCV and power density were optimized after a day of immersion in a 150 mM fructose solution with 1 mm electrode spacing. Following the charging period, the fuel cell functioned continuously for one week with a 65% reduction in the originally produced power density.

ACKNOWLEDGEMENT

The authors express their sincere appreciation to the Researchers Supporting Project Number (RSPD2025R679), King Saud University, Riyadh, Saudi Arabia.

REFERENCES

[1] Wang, Sijie, Wen Chen, Xinyue Huang, Xuezheng Chen, De Li, Feng Yu, and Yong Chen. 2023. "A Free-Standing Polymer Polypyrrole/Cellulose Composite Film via Spatial-Confined Interfacial Electrodeposition for Flexible Supercapacitors" Materials 16, no. 19: 6476. DOI: 10.3390/ma16196476

[2] Zhou-Rui, Li Dan-Dan, Kim Tae-Hee, Kim Min-Seok, Xu Ting, Ma Ming-Guo, Choi Sun-Eun, Si Chuanling, 2022, "Lignin-Based/Polypyrrole Carbon Nanofiber Electrode With Enhanced Electrochemical Properties by Electrospun Method," *Front Chem*, vol. 10, Feb doi: 10.3389/fchem.2022.841956.

[3] Sugumar Mohanasundaram, Barinderjit Singh, Nitin Govindprabhu Suradkar, S. Venkatesa Prabhu, Gomadurai Chinnasamy, Mukesh Goel & Jamal M. Khaled, 2023, "Extraction of pectin from Ethiopian prickly pear fruit peel and its potency for preparing of cellulose-reinforced biofilm," *Biomass Convers Biorefin*. DOI: 10.1007/s13399-023-04996-y

[4] J. P. Jyothibasu and R.-H. Lee, 2018, "Facile, Scalable, Eco-Friendly Fabrication of High-Performance Flexible All-Solid-State Supercapacitors," Polymers (Basel), vol. 10, no. 11. DOI: 10.3390/polym10111247

[5] Chen, Yasong, Fuying Wang, Lipan Dong, Zheng Li, Li Chen, Xinhai He, Jixian Gong, Jianfei Zhang, and Qiujin Li. 2019. "Design and Optimization of Flexible Polypyrrole/Bacterial Cellulose Conductive Nanocomposites Using Response Surface Methodology" Polymers 11, no. 6: 960. DOI: 10.3390/polym11060960

[6] S. Uzunçar, N. Özdoğan, and M. Ak, 2021, "Amperometric detection of glucose and H2O2 using peroxide selective electrode based on carboxymethylcellulose/polypyrrole and Prussian Blue nanocomposite," Mater Today Commun, vol. 26, p. 101839. DOI: 10.1016/j.mtcomm.2020.101839

[7] V. A. Nguyen, J. Wang, and C. Kuss, 2020, "Conducting polymer composites as water-dispersible electrode matrices for Li-Ion batteries: Synthesis and characterization," Journal of Power Sources Advances, vol. 6, p. 100033. DOI: 10.1016/j.powera.2020.100033

[8] T. Yuan, Z. Zhang, Q. Liu, X.-T. Liu, Y.-N. Miao, and C. Yao, 2023, "MXene (Ti3C2Tx)/cellulose nanofiber/polyaniline film as a highly conductive and flexible electrode material for supercapacitors," Carbohydr Polym, vol. 304, p. 120519. DOI: 10.1016/j.carbpol.2022.120519

[9] K. L. Knoche, D. P. Hickey, R. D. Milton, C. L. Curchoe, and S. D. Minteer, 2016 "Hybrid Glucose/O2 Biobattery and Supercapacitor Utilizing a Pseudocapacitive Dimethylferrocene Redox Polymer at the Bioanode," ACS Energy Lett, vol. 1, no. 2, pp. 380–385. DOI: 10.1021/acsenergylett.6b0022

[10] Thiyagarajan, K., Jayaraman, M., Vijayan, V., Ramkumar, R. (2020). Cluster analysis of lost foam casted Al-Zn-Mg-Cu alloy with K–Mean algorithm. Journal of New Materials for Electrochemical Systems, Vol. 23, No. 1, pp. 45-51. DOI: 10.14447/jnmes.v23i1.a09

[11] Goomin Kwon, Se-Hyun Kim, Dabum Kim, Kangyun Lee, Youngho Jeon, Cheon-Seok Park, Jungmok You, 2021, "Vapor phase polymerization for electronically conductive nanopaper based on bacterial cellulose/poly(3,4-ethylenedioxythiophene)," Carbohydr Polym, vol. 257, p. 117658. DOI: 10.1016/j.carbpol.2021.117658

[12] Sathish, T., Chandramohan, D., Vijayan, V., Sebastian, P.J. (2019). Investigation on microstructural and mechanical properties of Cu reinforced with Sic composites prepared by microwave sintering process. Journal of New Materials for Electrochemical Systems, Vol. 22, No. 1, pp. 5-9. DOI:10.14447/jnmes.v22i1.a02

[13] Michal Kizling, Krzysztof Stolarczyk, Petter Tammela, Zhaohui Wang, Leif Nyholm, Jerzy Golimowski, Renata Bilewicz, 2016, "Bioelectrodes based on pseudocapacitive cellulose/polypyrrole composite improve performance of biofuel cell," Bioelectrochemistry, vol. 112, pp. 184–190. DOI: 10.1016/j.bioelechem.2016.01.004

[14] A. Pizzariello, M. Stred'ansky, and S. Miertuš, 2002, "A glucose/hydrogen peroxide biofuel cell that uses oxidase and peroxidase as catalysts by composite bulk-modified bioelectrodes based on a solid binding matrix," Bioelectrochemistry, vol. 56, no. 1, pp. 99–105. DOI: 10.1016/S1567-5394(02)00026-9

[15] A. Karimi, A. Othman, A. Uzunoglu, L. Stanciu, and S. Andreescu, 2015, "Graphene based enzymatic bioelectrodes and biofuel cells," Nanoscale, vol. 7, no. 16, pp. 6909–6923. DOI: 10.1039/C4NR07586B

[16] Sugumar Mohanasundaram and Venkatramanan Varadharajan and Mayakannan Selvaraju and S Manikandan and Ramasamy Subbaiya and Mani Jayakumar and Venkatesa Prabhu Sundaramurthy and Baskar Gurunathan and Arivalagan Pugazhendhi, 2024, "Green ammonia as peerless entity for realm of clean-energy carrier toward zero carbon emission: Purviews, neoteric tendencies, potentialities and downsides," Fuel, vol. 365, p. 131118, 2024, doi: https://doi.org/10.1016/j.fuel.2024.131118.

[17] Periyasamy, Selvakumar search by orcid ; Asefa Adego, Adane ; Kumar, P. Senthil ; Desta, G. G. ; Zelalem, T. ; Karthik, V. ; Isabel, J. Beula ; Jayakumar, Mani search by orcid ; Sundramurthy, Venkatesa Prabhu ; Rangasamy, Gayathri, 2024, "Influencing factors and environmental feasibility analysis of agricultural waste preprocessing routes towards biofuel production – A review," Biomass Bioenergy, vol. 180, p. 107001. DOI: 10.1016/j.biombioe.2023.107001

[18] Manoj Kumar Sharma, S. Bhuvaneswari, Hitendra Kumar Lautre, Venkatesa Prabhu Sundramurthy, Sugumar Mohanasundaram, Jamal M. Khaled & Muthu Thiruvengadam, 2023, “Cellulose fortified bio-composite film preparation using starch isolated from waste avocado seed: starch properties and film performance,” Biomass Convers Biorefin, pp. 1–18. DOI: 10.1007/s13399-023-05058-z

[19] S. Yin, Z. Jin, and T. Miyake, 2019, “Wearable high-powered biofuel cells using enzyme/carbon nanotube composite fibers on textile cloth,” Biosens Bioelectron, vol. 141, p. 111471. DOI: 10.1016/j.bios.2019.111471

[20] Prashant Mishra, G.B.V.S. Lakshmi, Sachin Mishra, D.K. Avasthi, Hendrik C. Swart, Anthony P.F. Turner, Yogendra K. Mishra, Ashutosh Tiwari, 2017, “Electrocatalytic biofuel cell based on highly efficient metal-polymer nano-architectured bioelectrodes,” Nano Energy, vol. 39, pp. 601–607. DOI: 10.1016/j.nanoen.2017.06.023

[21] J. Filip, J. Šefčovičová, P. Gemeiner, and J. Tkac, 2013, “Electrochemistry of bilirubin oxidase and its use in preparation of a low cost enzymatic biofuel cell based on a renewable composite binder chitosan,” Electrochim Acta, vol. 87, pp. 366–374. DOI: 10.1016/j.electacta.2012.09.054

[22] M. Zhou and S. Dong, 2011, “Bioelectrochemical Interface Engineering: Toward the Fabrication of Electrochemical Biosensors, Biofuel Cells, and Self-Powered Logic Biosensors,” Acc Chem Res, vol. 44, no. 11, pp. 1232–1243. DOI: 10.1021/ar200096g

[23] Xin Li, Pengfei Lv, Yixin Yao, Quan Feng, Alfred Mensah, Dawei Li, Qufu Wei, 2020, “A novel single-enzymatic biofuel cell based on highly flexible conductive bacterial cellulose electrode utilizing pollutants as fuel,” Chemical Engineering Journal, vol. 379, p. 122316. DOI: 10.1016/j.cej.2019.122316

[24] I. Jeerapan, J. R. Sempionatto, and J. Wang, 2020, “On-Body Bioelectronics: Wearable Biofuel Cells for Bioenergy Harvesting and Self-Powered Biosensing,” Adv Funct Mater, vol. 30, no. 29. DOI: 10.1002/adfm.201906243

[25] J. H. Yang, H. R. Kim, J. H. Lee, J.-H. Jin, H. U. Lee, and S. W. Kim, 2021, “Electrochemical properties of enzyme electrode covalently immobilized on a graphite oxide/cobalt hydroxide/chitosan composite mediator for biofuel cells,” Int J Hydrogen Energy, vol. 46, no. 4, pp. 3251–3258. DOI:10.1016/j.ijhydene. 2020.03.084

[26] Y. Hui, H. Wang, W. Zuo, and X. Ma, 2022, “Spider nest shaped multi-scale three-dimensional enzymatic electrodes for glucose/oxygen biofuel cells,” Int J Hydrogen Energy, vol. 47, no. 9, pp. 6187–6199. DOI: 10.1016/j.ijhydene.2021.11.210

[27] H. Wu, Y. Zhang, A. Kjøniksen, X. Zhou, and X. Zhou, 2021, “Wearable biofuel cells: advances from fabrication to application,” Adv Funct Mater, vol. 31, no. 48, p. 2103976. DOI: 10.1002/adfm.202103976

[28] S. Alsaoub, F. Conzuelo, S. Gounel, N. Mano, W. Schuhmann, and A. Ruff, 2019, “Introducing pseudocapacitive bioelectrodes into a biofuel cell/biosupercapacitor hybrid device for optimized open circuit voltage,” ChemElectroChem, vol. 6, no. 7, pp. 2080–2087. DOI: 10.1002/celc.201900256

[29] Viktor Andoralov, Magnus Falk, Dmitry B. Suyatin, Marcus Granmo, Javier Sotres, Roland Ludwig, Vladimir O. Popov, Jens Schouenborg, Zoltan Blum & Sergey Shleev, 2013, “Biofuel cell based on microscale nanostructured electrodes with inductive coupling to rat brain neurons,” Sci Rep, vol. 3, no. 1, p. 3270. DOI: 10.1038/srep03270

[30] S.Dinesh, A.Godwin Antony, S.Karuppusamy, V.Vijayan and B.Suresh Kumar, 2016. Experimental investigation and optimization of machining parameters in CNC turning operation of duplex stainless steel. Asian Journal of Research in Social Sciences and Humanities 6,pp. 179-195. DOI: 10.5958/2249-7315.2016.01006.6

[31] S.Dinesh, A.Godwin Antony, K.Rajaguru and V.Vijayan. 2016. Experimental investigation and optimization of material removal rate and surface roughness in CNC turning of EN24 alloy steel, Mechanics and Mechanical Engineering, 20 (4) 451—466. DOI: 10.5958/2249-7315.2016.00654.7

[32] T.Tamizharasan, N.Senthil Kumar, V.Selvkumar, S.Dinesh, 2019. Taguchi’s Methodology of optimizing turning parameters over chip thickness ratio in machining PM AMMC, SN Appl. Sci. 1: 160., Springer Publishers. DOI: 10.1007/s42452-019-0170-8