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Influence of Buffer Electrolyte and pH on the Electrochemical Performance of Glucose Oxidase-Laccase Biofuel Cell

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We investigate the influence of buffer electrolyte and pH on the electrochemical performance of glucose oxidase-laccase biofuel cell (BFC). A simplified system design is adopted i.e., we employ freely-suspended glucose oxidase and laccase enzymes. The BFC comprises nickel mesh as the oxidative current collector and a carbon-based air electrode as the reductive current collector, enclosed in acrylic casing of 3 ml volumetric capacity. The air electrode also serves as the ambient oxygen diffusion site to continuously feed oxygen into the system. Three types of anolyte/catholyte buffer electrolyte are studied—citrate/citrate, phosphate/phosphate and citrate/phosphate, in the pH range 5–6.5. A biocatalytic electrochemical system is highly sensitive. Consequently, any variation in the electrolyte formulation would affect the cell discharge performance. Thus, we utilize the discharge profile capacity of the BFC to elucidate the optimum electrolyte formulation. Though the approach is indirect, the observed changes are obvious, suggesting the method is viable. It is found that the citrate/citrate buffer electrolyte of pH 5 exhibits the highest power output of 1074 μW h or volumetric power density of 1.7 W h l^{-1}. The cell is able to sustain continuous discharge current of 30 μA for about 31.75 hours with operating voltage around 1.0 V, in an uncontrolled open environment. Interestingly, the observed discharge performance and power density are comparable to BFC employing immobilized enzymes and mediators, in a controlled environment.

Keywords: Buffer Electrolyte, pH, Glucose Oxidase-Laccase Biofuel Cell.

1. INTRODUCTION

One of the major fields of research in the quest towards a clean energy substitute for fossil fuel is the research and development in biofuel cells (BFC).[1] BFC is an electrochemical system that generates electrical energy as a result of biocatalytic oxidation and reduction of organic fuel reactants. Thus it is a clean, non-polluting, renewable energy source and produces benign by-products as energy is released.

Enzymes, as functional proteins, are hypersensitive to the surrounding conditions. Furthermore, enzymes excreted by different microbes or even varying growth conditions often resulted in distinct characteristics.[2–4] Thus, in developing an enzymatic BFC, one of the critical parameters is to screen the optimum buffer electrolyte and pH in order to obtain the highest biocatalytic activities. The common approach was based on the individual laccase assay or half cell reaction.[5,6] Since the biocatalytic electrochemical system is highly sensitive, any variation in the electrolyte formulation would affect the overall cell discharge performance. As such, the present work attempted to elucidate the optimum biocatalytic activities of a glucose oxidase/laccase BFC based on the cell discharge performance. Glucose oxidase/laccase BFC is one of the most studied bioelectrochemical systems due to the high abundance of glucose in nature and the role of oxygen as the fuel oxidant.[7]

The overall electrochemical reactions of the glucose oxidase/laccase BFC can be summarized as follow

$$C_6H_{12}O_6 + 6H_2O \xrightarrow{GOx} 6CO_2 + 24H^+ + 24e^- \quad (1)$$

$$6O_2 + 24e^- + 12H_2O \xrightarrow{laccase} 24OH^- \quad (2)$$

In the system, glucose oxidase (GOx) enzyme catalyzes the oxidation of glucose fuel into gluconolactone while laccase enzyme catalyzes the reduction of oxygen, either in

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the dissolved form in the electrolyte or in the gaseous form at the triple gas–liquid–solid phase of the air electrode.

2. EXPERIMENTAL DETAILS

The BFC comprised of nickel mesh as the oxidative current collector and a carbon-based air electrode as the reductive current collector, enclosed in acrylic casing of 3 ml volumetric capacity. The air electrode also serves as the ambient oxygen diffusion site to continuously feed oxygen into the system. Figure 1 illustrates the schematic drawing of the BFC. The anolyte consisted of glucose oxidase enzyme (10 U) from *Aspergillus niger* (Biobasic Inc.), glucose substrate (200 mM) and FAD coenzyme (3.8 mM), while the catholyte consisted of laccase enzyme (10 U) from *Rhus vernificera* (Sigma-Aldrich) and syringaldazine substrate (216 μM). Syringaldazine in the catholyte serves as an enhancer to catalytic reduction of oxygen. Laccase stores electrons from syringaldazine oxidation which are then utilized to reduce molecular oxygen. A cellulose ionic exchange membrane (Futamura Chemical Co. Ltd., Japan) was utilized to separate the anolyte and catholyte. In this work, we employed freely-suspended glucose oxidase and laccase enzymes in the buffer electrolyte i.e., not immobilized. This simplified design was adopted for rapid optimization approach.

We investigated the electrochemical performance of glucose oxidase-laccase BFC utilizing 3 types of anolyte/catholyte buffer electrolyte—citrate/citrate, phosphate/phosphate and citrate/phosphate, in the pH range 5–6.5. The choice of buffer electrolyte and pH range was based on commonly reported electrolyte formulations in the literature.[8,9] Our conjecture was any small variation in the electrolyte formulation would result in a distinct discharge profile capacity. Thus, the BFC was gauged directly form its continuous discharge capacity at 30 μA. All measurements were performed using an Eco Chemie Autolab Potentiostat/Galvanostat (PGSTAT302N) and conducted at room temperature of 30 °C in an open, uncontrolled ambient environment.

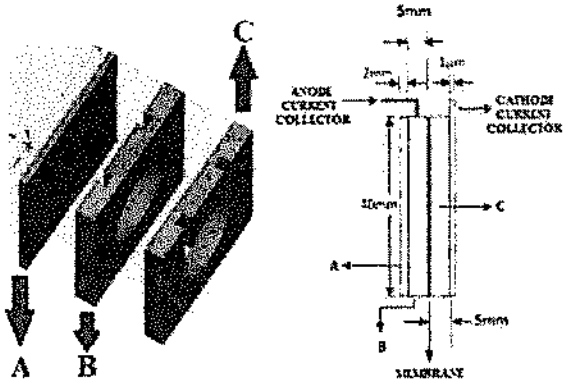

Fig. 1. BFC cell design.

3. RESULTS AND DISCUSSION

We adopted a factorial design optimization. Based on 3 types of buffer electrolyte and 3 pH values, there were a total of 15 electrolyte formulations as listed in Table I. Unlike most studies in BFC, we screened the electrolyte formulation directly from the electrochemical performance of a complete cell rather than based on individual enzyme assay or half cell reaction. Figures 2 and 3 depict the variation of total power gain of the BFC as a function of electrolyte formulation. The total power gain was calculated from the product of discharge current and the area under the discharge plot

$$\text{Power gain, } P = I_d \int V(t)\,dt = I_d A_n \text{ (W h)} \quad (3)$$

where I_d is the constant discharge current, A_n is the area under the discharge plot, $V(t)$ is the cell's operating

Table I. Electrolyte formulations tested for the glucose oxidase-laccase BFC.

Formulation	Anolyte	Catholyte
	Citrate buffer (pH)	
A		
1	5	
2	5.7	
3	6.5	
	Phosphate buffer (pH)	
B		
4	5	
5	5.7	
6	6.5	
	Citrate buffer (pH)	Phosphate buffer (pH)
C		
7	5	5
8	5.7	5
9	6.5	5
10	5	5.7
11	5.7	5.7
12	6.5	5.7
13	5	6.5
14	5.7	6.5
15	6.5	6.5

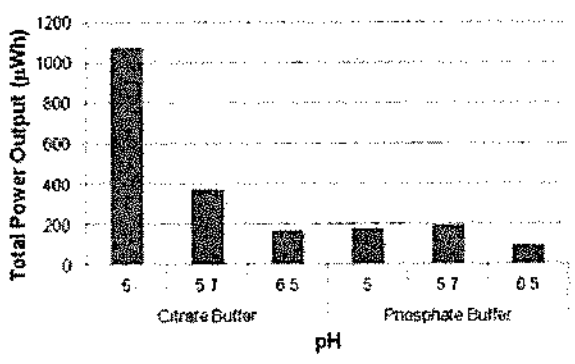

Fig. 2. Plot of BFC power output gain against electrolyte formulation (both anolyte and catholyte employing either citrate or phosphate buffer).

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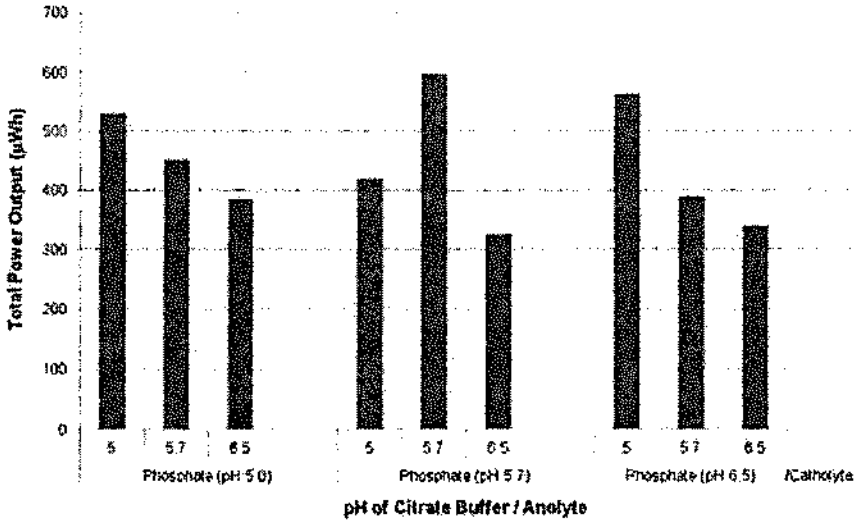

Fig. 3. Plot of BFC power output gain against electrolyte formulation (anolyte in citrate buffer and catholyte in phosphate buffer).

voltage. A_n was estimated from the Riemann's Sum approximation,

$$A_n = \sum_{i=1}^{n} \Delta x f(x_i) \quad (2)$$

where n is the total data points, Δx is the interval between data points, and $f(x_i)$ is the right end point of the ith interval.

As anticipated, variation in the electrolyte formulation produced distinct discharge performance. Glucose oxidase-laccase BFC employing citrate buffer pH 5 possessed the highest power output i.e., 1074 μW h or volumetric power density of 1.7 W h l^{-1}, while the BFC using phosphate buffer seemed not favourable for glucose oxidase catalytic activity.

Figure 4 illustrates the discharge profiles of the BFC based on the best profile obtained from electrolyte formulation A, B and C (refer to Table 1). The most prevalent feature of citrate buffer pH 5 formulation as compared to customary citrate/phosphate formulation was its high operating voltage, around 1.0 V. Although the cell was able to sustain continuous current drain of 30 μA for nearly 57 hours, most of the extra discharge duration was of low voltage. The observed results also suggest that the BFC employing citrate buffer for glucose oxidase and phosphate buffer for laccase appeared to be more

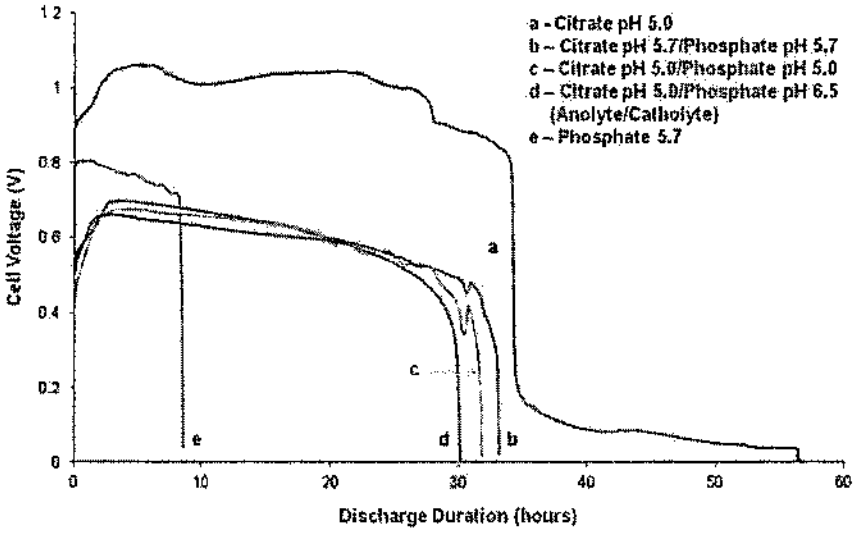

Fig. 4. Discharge capacity profiles of BFC with different electrolyte formulations.

tolerant to pH variation, as indicated by the more or less compatible discharge performance (profiles b, c and d). Finally, it is interesting to note that the observed discharge performance and power density are compurable to BFC employing immobilized enzymes and mediators, as well as operated in controlled environment.[8,16]

4. CONCLUSION

We have screened the optimum electrolyte formulation, namely buffer electrolyte and pH, of a glucose oxidase/laccase BFC utilizing directly the discharge capacity of the cell, rated at 30 μA. As the biocatalytic electrochemical system is inherently very sensitive, any variation in the electrolyte formulation would produce distinct discharge profile. Although the approach is indirect, the observed changes are obvious, suggesting the method is viable. It is observed that the glucose oxidase/laccase BFC employing citrate/citrate buffer electrolyte of pH 5 possessed the optimum power output of 1074 μW h or volumetric power density of 1.7 W h l^{-1}.

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