Improving the performance of lactate/oxygen biofuel cells using a microfluidic design

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ABSTRACT

Lactate/O₂ biofuel cells (BFC) can have high theoretical energy densities due to high solubility and high fuel energy density; however, they are rarely studied in comparison to glucose BFCs. In this paper, lactate oxidase (LOx) was coupled with a ferrocene-based redox polymer (dimethylferrocene-modified linear polyethylenimine, FcMe₂-LPEI) as the bioanode and laccase (Lc) connected to pyrene-anthracene modified carbon nanotubes (PyrAn-MWCNT) to facilitate the direct electron transfer (DET) at the biocathode. Both electrodes were evaluated in two BFC configurations using different concentrations of lactate, in the range found in sweat (0-40 mM). A single compartment BFC evaluated at pH 5.6 provided an open circuit potential (OCP) of 0.68 V with a power density of 61.2 μW cm⁻². On the other hand, a microfluidic BFC operating under the same conditions resulted in an OCP of 0.67 V, although an increase in the power density, increasing to 305 μW cm⁻², was

observed. Upon changing the pH to 7.4 in only the anolyte, its performance was further increased to 0.73 V and $404 \mu\text{W cm}^{-2}$, respectively. This work reports the first microfluidic lactate/oxygen enzymatic BFC and shows the importance of microfluidic flow in high performing BFCs where lactate is utilized as the fuel and O_2 is the final electron acceptor.

Keywords: lactate; microfluidic; biofuel cells; air-breathing.

1. Introduction

Lactate is an intermediate metabolite of anaerobic glycolysis and is present mainly in blood, serum, tears and sweat. There has been interest in the quantification of blood lactate due to its association with severe illnesses such as pneumonia, congestive heart diseases, coronary artery disease and diabetic coma; in the analysis of food as the evaluation of the freshness and stability of milk, dairy products, fruits, vegetables, meats and wines; in biotechnological processes; and to monitor high-performance athletes [1]. In recent years, electrochemical methods for lactate sensing using amperometry have become popular due to their versatility, sensitivity, reproducibility and ease of miniaturization. These lactate biosensors are based on monitoring either the concentration of O_2 or H_2O_2 and usually the electrodes are based on immobilized enzymes such as lactate dehydrogenase (LDH)[2], cytochrome b₂ (Cyt b₂)[3], and lactate-2-monooxygenase or lactate oxidase (LOx)[4]. LOx is the enzyme most widely used and its flavin adenine dinucleotide-dependent (FAD) general reaction mechanism sees the oxidation of *L*-lactate to pyruvate coupled to the reduction of O_2 to H_2O_2 (Figure 1) [5].

Figure 1: General reaction of the oxidation of lactate by the enzyme LOx.

Lactate is an interesting fuel for BFCs, because it is readily available in biological fluids for implantable applications and it has a high energy density for portable power applications. The oxidation of lactate by a single enzyme (*i.e.*, LDH) could generate a theoretical energy density of up to 507 Wh/L at 7.6M lactate and the complete oxidation of lactate could theoretically result in a maximum energy density of 3041 Wh/L [6]. Thus, lactate is an interesting fuel source for alternative energy generation.

A microfluidic biofuel cell (μ BFC) can be defined as a BFC where the delivery of fuel and removal of waste takes place via a microfluidic channel incorporating electrode structures. These devices typically do not require a physical barrier (such as a Nafion membrane) to separate the anodic and cathodic compartments. One type of μ BFC is a flow-through device that consists of porous electrodes where the fuel and oxidant flow through the electrodes to improve fuel utilization [7]. The implementation of flow-through μ BFC technologies allow for higher enzyme loadings and high surface area to volume ratios to promote the surface-based electrochemical reactions catalyzed by the immobilized enzymes [8]. Although μ BFCs have been evaluated for glucose [9] and ethanol [10] mainly, they have never evaluated the use of lactate as fuel, even though lactate is a common fuel in biological samples and has high energy density. This paper combines the advantages of μ BFCs with the high energy densities made available by lactate/ O_2 BFC systems for improving the performance of biofuel cells.

2. Materials and methods

All chemicals were reagent grade and were used without further modification. Lactic acid was purchased from Sigma Aldrich. Lactate oxidase (LOx) was from *Aerococcus viridians* (Sekisui Diagnostics (UK), activity 41 U mg⁻¹). Ethylene glycol diglycidyl ether (EGDGE) was obtained from Polysciences, Inc. Laccase (Lc) was from *Trametes Versicolor* (activity 10 U mg⁻¹) and was acquired from Sigma Aldrich. Tetrabutylammonium bromide (TBAB)-modified Nafion was prepared as described previously [11].

2.1 Electrode preparation

For the characterization of lactate bioelectrocatalysis, LOx was immobilized on Toray carbon paper (TCP) with a geometric area of 0.25 cm² and a thickness of 190 μm. The redox polymer used was dimethylferrocene-modified linear polyethyleneimine (FcMe₂-LPEI), which was prepared as previously reported [12]. A solution of FcMe₂-LPEI (10 mg mL⁻¹ in deionized water (DI)) was mixed with 200 U mL⁻¹ of LOx (in PBS pH 7.4) and 2 – 6 % v/v of cross-linking agent (EGDGE) in DI, at a volumetric ratio of 56/24/3 and thoroughly mixed. Finally, 25 μL was pipetted onto each carbon electrode surface and allowed to dry overnight at room temperature.

Biocathodes were prepared by mixing a solution of Lc (20 mg mL⁻¹ in PBS at pH 6.5) with 7.5 mg of MWCNTs previously modified with anthracene [13] or pyrene-anthracene [14] and was mixed by 4 successive mixing steps of vortex-mixing (1 min) and sonication (15 s). TBAB-modified Nafion was added (25 μL) and a further single mixing step was

performed. Finally, 9 μL of the solution was deposited onto aTCP electrode using a brush and allowed to dry for 2 h under positive air flow.

2.2 Electrochemical methods

Electrochemical experiments were conducted using a CH Instruments potentiostat. $\text{FcMe}_2\text{-LPEI/LOx}$ electrodes were evaluated in phosphate buffer solution (PBS, pH 7.4, 50 mM) using a saturated calomel electrode (SCE) and platinum mesh as the reference and counter electrode, respectively. Cyclic voltammetry was performed at a scan rate of 2 mV s^{-1} and potential range of -0.1 to 0.5 V (vs. SCE), while the amperometric experiments were performed at a constant applied potential of 0.28 V (vs. SCE).

The comparison between An-MWCNT/Lc and PyrAn-MWCNT/Lc electrodes was performed *via* cyclic voltammetry at a range of potentials from 0.8 to 0 V (vs. SCE) and a scan rate of 1 mV s^{-1} in PBS (pH 5.6, 0.1 M) in air. Polarization curves for the BFC in a single compartment were achieved by linear polarization at 2 mV s^{-1} (0.083 and 2 mg cm^{-2} of LOx and Lc enzyme loading, respectively). The supporting electrolyte was 0.2 M PBS (pH 5.6) using different concentrations of lactate (0 , 2 , 10 , 20 and 40 mM). For the fuel cell evaluation, a μBFC with an air-breathing cathode was fabricated as previously reported [15]. Both plates were made using PMMA [poly-(methyl methacrylate)] with a silicon elastomer film (Silastic[®]) compressed between the plates used to seal the device and define the channel pattern. In the top plate, an air-intake window was constructed with a dimension of 0.22 cm^2 enabling oxygen delivery from air. The anodes and cathodes used were derived of $\text{FcMe}_2\text{-LPEI/LOx}$ and PyrAn-MWCNT/Lc , respectively, immobilized onto TCP (Scheme 1) with a dimension of $3 \text{ cm} \times 0.2 \text{ cm}$ and placed above the bottom plate (1.034 and 2.5 mg cm^{-2} of LOx and Lc enzyme loading respectively). All reported current densities have been

normalized to the electrode cross-sectional area perpendicular to the flow direction (0.02 cm^2). The flow rate of the stream of both anolyte and catholyte for μBFC tests was controlled using a syringe pump and the rate was held at 3 mL h^{-1} , except for the evaluation of the μBFC at different flow rates of 1, 3, 6, 9 and 12 mL h^{-1} .

Scheme 1:(Top)Main μBFC components including an air-breathing cathode and top and bottom Poly-methyl methacrylate (PMMA) supports. Inset: photograph of

μ BFC.(Bottom)Scheme of flow-through electrodes concept in air-breathing membraneless microfluidic fuel cell.

3. Results and discussion

3.1 Characterization of the $\text{FcMe}_2\text{-LPEI/LOx}$ bioanode

The $\text{FcMe}_2\text{-LPEI/LOx}$ bioanode was characterized by examining the quasi-reversible redox peaks that belong to the electron mediator, dimethylferrocene (FcMe_2). In Figure 2, cyclic voltammetry shows the redox couple FcMe_2 on TCP with oxidation and reduction peak potentials of 0.28 and 0.16 V (vs. SCE), respectively (dashed line). In the presence of 70 mM lactate, the limiting current density (j_{max}) was $213 \mu\text{A cm}^{-2}$ obtained for the mediated oxidation of lactate by LOx (solid line, determined at a potential of 0.5 V).

Figure 2: Representative cyclic voltammograms of $\text{FcMe}_2\text{-LPEI/LOx}$ immobilized on TCP in the absence (dashed line) and the presence (solid line) of 70 mM lactate pH 7.4 PBS at a scan rate of 2 mV s^{-1} .

The catalytic kinetics of the LOx bioanode were studied by amperometry with the addition of different concentrations of lactate (Figure 3A). The resulting calibration curve for lactate

detection demonstrates a similar current density of $204.1 \mu\text{A cm}^{-2}$ using 70 mM lactate and an apparent Michaelis constant (K_M^{app}) of $8.9 \pm 1.1 \text{ mM}$ calculated by double-reciprocal evaluation of the Lineweaver-Burk equation. Depending on the method of immobilization and the enzyme, K_M values have been reported ranging from 0.2 mM to 16.7 mM for LOx or LDH [16]. The limit of detection calculated for lactate was 0.091 mM and the limit of quantification was determined to be 0.27 mM. The sensitivity of the amperometric sensor was $14.4 \mu\text{A mM}^{-1} \text{ cm}^{-2}$ ($r^2=0.9974$). Additionally, the linear response range was 0 – 5 mM with a response time of 10 s (Figure 3B). These results show the effective catalytic activity of LOx with FcMe₂-LPEI as the mediator in the presence of lactate.

Figure 3.(A) Amperometric response and (B) the resulting calibration curve of FcMe₂-LPEI/LOx immobilized on TPC at various concentrations of lactate at an applied potential of 0.28 V (vs SCE) in pH 7.4 PBS.

3.2 Characterization of the PyrAn-MWCNT/Lcbiocathode

Two different modifications of MWCNTs based of anthracene (An-MWCNT) and pyrene-anthracene (PyrAn-MWCNT) were used for immobilizing the Lcon TCP, followed by a protective coating of TBAB-modified Nafion, in order to determine which of the two

modifications allows for a higher catalytic activity of the oxygen reduction reaction (ORR). Although these two modified MWCNTs have been previously reported in the literature [13, 14], a direct comparison between them was needed to establish which would yield the most suitable biocathode for this particular biofuel cell configuration. The experiments were performed in 0.1 M PBS at pH 5.6 and cyclic voltammograms are shown in Figure 4. The total current density was determined from background subtraction between 0.61 V and 0 V (vs. SCE). The cyclic voltammograms for An-MWCNT/Lc electrodes or PyrAn-MWCNT/Lc electrodes in nitrogen-saturated buffer did not yield a catalytic current for the ORR (dotted line). Upon evaluation in air-saturated buffer, the An-MWCNT/Lc electrodes resulted in a current density of $68 \mu\text{A cm}^{-2}$ without stirring (dashed line), while PyrAn-MWCNT/Lc electrodes yielded a current density of $111.3 \mu\text{A cm}^{-2}$ (solid line). Therefore, we utilized the PyrAn-MWCNT/Lc electrodes for BFC experiments. It is important to note that the anthracene is a mimic for the natural substrate of the enzyme minimizing the distance between the T1 Cu site of the enzyme and the carbon nanotube and facilitating DET [17], but the An-MWCNT only have anthracene functional groups on the end of the tubes, while the aromatic pyrene of PyrAn-MWCNT can interact directly with the walls of the MWCNT via π -stacking; thereby, increasing the loading of anthracene on the nanotubes and improving the dispersal of MWCNT [18]. Therefore, the current density value was higher with PyrAn-MWCNT/Lc than An-MWCNT/Lc, as expected from previous literature [13,14].

Figure 4. Representative cyclic voltammograms comparing cathodes made from anthracene-modified MWCNT (dash) and anthracene-modified pyrenes immobilized on

MWCNT (solid) with Lc on TCP without stirring under air-saturated 0.1 M PBS pH 5.6 at a scan rate of 1 mV s⁻¹ and N₂-saturated (dot).

3.3 Evaluation of a lactate/O₂BFC in a single compartment

The evaluation of the BFC in a single compartment (SC) was performed in 0.2 M PBS pH 5.6 under different physiologically relevant concentrations of lactate (0, 2, 10, 20, and 40 mM) at 25 °C. Both the bioanodes with FeMe₂-LPEI/LOx and the biocathodes with PyrAn-MWCNT/Lc covered with TBAB-modified Nafion were utilized in a single compartment (membraneless) BFC with both electrode areas being 0.25 cm². Polarization curves were produced by linear polarization at a scan rate of 2 mV s⁻¹. As shown in Table 1, the open circuit potential (OCP) was maintained at an average of 0.64 ± 0.03 V for the different concentrations of lactate, with the exception of 40 mM lactate, which resulted in a decreased OCP of 0.47 ± 0.05 V. The current and power densities obtained were 284 ± 17 μA cm⁻² and 61.2 ± 9.2 μW cm⁻², respectively. These are higher than those reported for lactate dehydrogenase of 153 μA cm⁻² and 44 μW cm⁻² [6].

	OCP (V)	J/ μA cm ⁻²	W/ μW cm ⁻²
[lactate] / mM			
0	0.63	223	29.7
2	0.62	240	42.7
10	0.68	272	48.8
20	0.62	284	61.2
40	0.47	121	21.8

Table 1. Performance of the single compartment BFC including the FeMe₂-LPEI/LOx anode and the PyrAn-MWCNT/Lc cathode using different concentrations of lactate.

3.4 Evaluation of an air-breathing lactate/O₂ microfluidic biofuel cell (μBFC)

The operating conditions for the μBFC were the same as for the single compartment BFC except that the PBS/lactate solution was pumped through the electrodes inside the device

shown in Scheme 1. Table 2 reports OCPs, current densities and power densities for the μ BFCs, where the largest OCPs were 0.63 ± 0.04 and 0.64 ± 0.02 V using either 2 or 10 mM lactate, respectively. Such OCPs are comparable to the single-compartment BFCs, although the current and power densities were $1755 \pm 51 \mu\text{A cm}^{-2}$ and $188 \pm 13 \mu\text{W cm}^{-2}$, with a decrease at higher concentrations. The higher current density reached with the μ BFC is mainly due to the air-breathing system, because the diffusion coefficient of oxygen in air ($0.2 \text{ cm}^2 \text{ s}^{-1}$) is higher than in aqueous media ($2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$) and the oxygen concentration available in the air-breathing μ BFC is higher and constant [19].

	OCP (V)	J/ $\mu\text{A cm}^{-2}$	W/ $\mu\text{W cm}^{-2}$
[lactate] / mM	Toray		
0	0.52	1241	181
2	0.64	1755	181
10	0.63	1186	188
20	0.55	1077	188
40	0.45	973	141

Table 2. Performance of the μ BFC using a 0.2 M PBS at pH 5.6 and 25 °C.

Previously, we have improved the performance of glucose μ BFCs by decreasing the concentration of both supporting electrolyte to 0.05 M [20]. In order to improve the performance of the lactate μ BFCs, we employed this same strategy. With these changes to the electrolyte concentration we obtained an OCP of 0.65 ± 0.03 V, achieving a maximum OCP of 0.67 V using 20 and 40 mM of lactate. Also, the current and power densities increased considerably with the concentration of lactate, achieving $1700 \pm 77 \mu\text{A cm}^{-2}$ and $305 \pm 20 \mu\text{W cm}^{-2}$ using 40 mM lactate, respectively (Table 3-left). The performance of the μ BFC using 0.05 M PBS was higher and more stable than under the original concentration of PBS. In order to take advantage of the μ BFC design that allows the use of individual electrolyte inlets for the anode and cathode, the pH of the anolyte was changed to pH 7.4 (0.05 M), as appropriate for use in physiological conditions, and the pH of the catholyte was kept at 5.6 (0.05 M), since voltammetry shows very little oxygen bioelectrocatalysis at pH 7.4, as shown in Figure 5. The OCP increased to 0.73 V and remained constant at concentrations ranging from 2 to 40 mM of lactate, achieving maximum current and power densities of $2087 \pm 84 \mu\text{A cm}^{-2}$ and $402 \pm 23 \mu\text{W cm}^{-2}$ using 10

mM of lactate. This is a 19% and 24% enhancement in performance compared to employing the same pH (Table 3-right).

FeMe ₂ -LPEI/LOx	OCP (V)	J/ $\mu\text{A cm}^{-2}$	W/ $\mu\text{W cm}^{-2}$	OCP (V)	J/ $\mu\text{A cm}^{-2}$	W/ $\mu\text{W cm}^{-2}$
[Lactate] / mM	pH 5.6			pH 7.4		
0	0.61	1510	235	0.67	1607	249
2	0.66	1595	267	0.73	1893	333
10	0.66	1610	291	0.73	2087	404
20	0.67	1640	293	0.73	2040	392
40	0.67	1700	305	0.73	1920	364

Table 3. Performance of the μBFC using different concentrations of lactate in 0.05 M PBS at pH 5.6 (left) and change the pH of PBS in the anode to pH 7.4 (right).

Figure 5. Comparative voltammograms of biocathode PyrAn-MWCNT/Lc in 0.1 M PBS pH 5.6 (solid line) and pH 7.4 (dash line) at a scan rate of 1 mV s^{-1} .

In previous work by the Minter Researchgroup, FcMe₂-LPEI/LOx electrodes were evaluated at a pH range between 4.5 - 9.5. This data showed the FcMe₂-LPEI/LOx electrodes preserving 85% of its maximum catalytic activity at pH 7.4 while preserving only 45% of the maximum catalytic activity at pH 5.6. For this reason, we predicted that the μBFC could have a higher current density [24]. Figure 6 shows the polarization and power density curves comparison between different μBFC evaluated using 10 mM lactate highlighting the performance obtaining with μBFC in pH 7.4. Importantly, another

advantage of the μ BFC design is that it reduces the effects of fuel crossover as shown in our previous work [15], which could explain the decrease in the OCP observed for the single compartment BFCs. Also, the μ BFC design allowed for the operation of the device in the absence of a membrane, enabling the independent use of different electrolytes for the bioanode and biocathode which improves enzymatic catalytic activity. Further, the air-breathing system constantly delivers a high concentration of oxygen with lower transport limitations resulting in an increased current density.

Figure 6. Comparison of polarization and power density curves between different μ BFCs evaluated in different operating conditions using 10 mM lactate characterized at 2 mVs^{-1}

Also, the effect of flow rate was evaluated using a concentration of 10 mM lactate in 0.05M at pH 7.4 for the anolyte and pH 5.6 for the catholyte. The OCP of the μ BFC was 0.73 V. The results show that a flow rate of 3 mL h^{-1} allowed the OCP to reach a maximum in less time (6.9 min) compared to a flow rate of 9 mL h^{-1} which resulted in an improved current density of $1875 \mu\text{A cm}^{-2}$ although the average stabilization time was 11.2 min. Interestingly, the current density begins to decrease at a flow rate of 12 mL h^{-1} (Figure 7), perhaps due to flow-induced mechanical instabilities in the CNT immobilization.

Figure 7. Evaluation of the μ BFC at different flow rates of 1, 3, 6, 9 and 12 mL h⁻¹ in both the cathodic and anodic streams where blue is the OCP stabilization time and black is the current density.

Table 4 compares this reported μ BFC with other lactate enzymatic BFCs where the fuel is oxidized by LOx or LDH. Minteer's group recently reported the use of NAD-dependent LDH immobilized in TBAB-modified Nafion for lactate oxidation obtaining current and power densities of 153 μ A cm⁻² and 44 μ W cm⁻², where the same report used a cascade of 6 dehydrogenases for the complete oxidation of lactate which resulted in an increase of 21.7 and 18.8 times the current and power densities in a single compartment BFC [6]. The Wang research group used lactate produced in sweat as a BFC-type tattoo on the skin. They reported an OCP of 0.54 V and a power density of 44 μ W cm⁻² in a traditional BFC, but observed an increase in the power density to 55 – 70 μ W cm⁻² for use in the tattoo format [21]. On the other hand, Willner studied the effect of the oxidation of lactate by LDH either with or without a magnetic-field and reported an OCP of 0.12 V with a current density of 300 μ A cm⁻² [22]. Willner and collaborators also used a bioanode with LOx and GOx for the oxidation of lactate and glucose separately or simultaneously, while the cathode consisted of bilirubin oxidase and catalase for the reduction of oxygen and/or

H₂O₂. They reported a maximum density power of 42 $\mu\text{W cm}^{-2}$ for the oxidation of lactate only [23]. The Minter research group also recently reported lactate oxidation by LOx using the above ferrocene redox polymer (FcMe₂-LPEI) coupled with LOx, yielding an OCP of 0.57 V and a power density of 122 $\mu\text{W cm}^{-2}$ for a self-powered amperometric lactate biosensor [24]. In this work, we reported a comparative study between a BFC in a single compartment and microfluidic air-breathing mode operating in different conditions. The single compartment BFC reached a maximum OCP of 0.68 V while the μBFC OCP reached 0.73 V using separate anolyte and catholyte flows. Also, in this final optimized configuration, maximum current and power densities of 2087 $\mu\text{A cm}^{-2}$ and 404 $\mu\text{W cm}^{-2}$ were observed, which are higher than any single enzyme (LOx or LDH) lactate BFC in the literature. This demonstrates the advantages of a microfluidic platform in minimizing crossover to increase OCP and allowing the use of ionic strengths and pH that are more biocompatible for cathode and anode enzyme catalysis. Also, the air-breathing cathodic system allowed a constant oxygen concentration resulting in increased power and current densities.

Bioanode/anolyte	Biocathode/catholyte	OCP	$j/\mu\text{A cm}^{-2}$	$W/\mu\text{W cm}^{-2}$	Type BFC	Ref
LDH-TBAB-NAD/PBS pH 7.4, 100 mM Lac.	Platinum / PBS pH 7.4	NS	153	44	SC	[6]
6 dehydrogenases /PBS 7.4, 100 mM Lac.	Platinum / PBS pH 7.4	NS	3320	827	SC	[6]
LOx-TTF-CNT-Chit / 0.2 M Mcllvaine pH 5.5, 20 mM	Platinum-Nafion / 0.2 M Mcllvaine pH 5.5	0.54	NS	44	SC	[21]
LOx-TTF-CNT-Chit/ sweat	Platinum-Nafion / sweat	NS	NS	55 - 70	Skin	[21]
Magnetic-fiel-LDH-NAD-PQQ/0.1 M TRIS pH 7, 20 mM Lac.	COx-Cytc / 0.1 M TRIS pH 7 O ₂	0.12	300	12.4	SC	[22]
LOx-FeMeOH-CNPs-MB-/ 0.1 M HEPES pH7.0, 25 mM Lac.	BOD-CNPs-ABTS-Cat / 0.1 M HEPES pH7.0, O ₂ -H ₂ O ₂	NS	NS	30 (O ₂) 35 (O ₂ -H ₂ O ₂) 42 (H ₂ O ₂)	SC	[23]
FeMe ₂ -LPEI-LOx / 0.05 M PBS pH 6.5, 5 mM Lac.	An-MWCNT-BOD / 0.05 M PBS pH 6.5	0.57	657	122	SC	[24]
FeMe ₂ -LPEI-LOx / 0.2 M PBS pH 5.6, 10 mM Lac.	PyrAn-MWCNT-Lc / 0.2 M PBS 5.6	0.68	272	48.8	SC	This work
FeMe ₂ -LPEI-LOx / 0.2 M PBS 5.6, 2 mM	PyrAn-MWCNT-Lc / 0.2 M PBS 5.6	0.64	1755	181	μ BFC	This work
FeMe ₂ -LPEI-LOx / 0.05 M PBS pH 5.6, 40 mM Lac	PyrAn-MWCNT-Lc / 0.05 M PBS pH 5.6	0.67	1700	305	μ BFC	This work
FeMe ₂ -LPEI-LOx / 0.05 M PBS pH 7.4, 10 mM Lac	PyrAn-MWCNT-Lc / 0.05 M PBS pH 5.6	0.73	2087	404	μ BFC	This work
TTF=tetrathiafulvalene; Chit=chitosan; CNPs=carbon nanoparticles; MB=methyl blue; Cat=catalase; NS=Not specified; SC=single compartment; Cytc=cytochrome C; Cox=cytochrome oxidase						

Table 4. Comparative performance of lactate/air enzymatic BFCs reported in the literature.

4. Conclusions

This work showed the performance of a LOx-based lactate BFC evaluated in a single compartment and in a flow-through air-breathing μ BFC. Comparison of the two MWCNT-modified biocathodes both showed good ORR performance, however, the PyrAn-MWCNT/Lc modification produced higher current density than the An-MWCNT/Lc cathodes. FeMe₂-LPEI/LOx bioanodes and PyrAn-MWCNT/Lc biocathodes used in a single compartment BFC achieved a maximum OCP of 0.68 V and delivered a maximum current and power density of 272 $\mu\text{A cm}^{-2}$ and 48.8 $\mu\text{W cm}^{-2}$, respectively. However,

improved BFC performances were obtained with the μ BFC configuration, including an OCP of 0.73 V with a current and power density of $2087 \mu\text{A cm}^{-2}$ and $404 \mu\text{W cm}^{-2}$, respectively. This is the highest performing lactate/oxygen biofuel cell utilizing a single enzyme system. Future work will focus on incorporating multi-enzyme cascades to further improve performance.

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