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Performance Optimization of Microbial Fuel Cell (MFC) Using *Lactobacillus bulgaricus*

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Abstract

Electrical energy needs in Indonesia are expected to continue to rise. The use of petroleum as a source of energy still dominates, although oil reserves in Indonesia are increasingly being depleted. Therefore, there is a need to develop alternative sources of sustainable energy, such as microbial fuel cell (MFC). In this study, *Lactobacillus bulgaricus* was used as an electricity producer in a dual-chamber MFC reactor. We investigated the maximum electrical energy by varying the bacterial optical density (OD), the operational time of MFC, the reactor volume, the electrolyte solution, and the configuration of MFC reactor. In this study, the maximum electrical energy (201.8 mW/m^2) was generated at an OD of 0.5 in an MFC reactor series using potassium permanganate as the electrolyte solution.

Abstrak

Optimasi Kinerja *Microbial Fuel Cell* (MFC) dengan Bakteri *Lactobacillus bulgaricus*. Kebutuhan energi listrik di Indonesia diperkirakan akan terus meningkat. Namun penggunaan minyak bumi sebagai sumber penghasil energi masih mendominasi, padahal cadangan minyak bumi di Indonesia kian menipis. Oleh karena itu, perlu dikembangkan alternatif penghasil sumber energi yang berkelanjutan, salah satunya adalah *microbial fuel cell* (MFC). Pada penelitian ini, digunakan bakteri *Lactobacillus bulgaricus* sebagai penghasil listrik pada reaktor MFC *dual-chamber*. Untuk memperoleh energi listrik yang maksimum, dilakukan variasi *optical density* (OD), waktu operasi, volume reaktor, larutan elektrolit, dan konfigurasi reaktor MFC. Dari penelitian ini, dihasilkan energi listrik maksimum berupa *power density* sebesar 201,9 mW/m^2 pada reaktor MFC seri dengan OD 0,5 dan kalium permanganat sebagai larutan elektrolit.

Keywords: dual-chamber reactor, Lactobacillus bulgaricus, microbial fuel cell, power density

1. Introduction

The Electrical energy needs of Indonesia are estimated to continue to grow at 4.6% annually. This figure is expected to have trebled by 2030. If this is not accompanied by efforts to increase energy production, there is a risk that Indonesia will experience an energy crisis. The use of petroleum as an energy-producing fossil fuel still dominates. However the country only has around 3.7 billion barrels of petroleum. As the supply can be expected to be exhausted in 24 years, there is an urgent need to generate alternative energy sources (sustainable technology).

Microbial fuel cells (MFCs) which use bacteria to generate electricity from organic and nonorganic compounds are one prospective alternative technology. As noted in the study by Barua [1], bacteria are capable of producing electrical energy. Many other studies have examined the performance of MFCs in generating electrical energy. Min *et al*. [2] assessed the effect of temperature and anode media on electrical energy production. Li *et al*. [3] attempted to optimize MFCs for use in electricity production, studying the configuration of the MFC reactor, the type of electrolyte used and the type of electrode material used. Various types of microbes have been used as MFCs, such as *Geobacter sulfurreducens* [4], *Escherichia coli* [5], *Saccharomyces cerevisiae* [6], and *Shewanella oneidensis* [7].

We conducted an MFC performance optimization study using *Lactobacillus bulgaricus* in a dual-chamber reactor. We assessed the effect of the optical density (OD 0.5, 0.6, 0.7), the operation time (3, 30, and 100 hours), the reactor volume (100 and 500 mL), and the

type of electrolyte (potassium ferricyanide and potassium permanganate) on the performance of the MFC system. The maximum value of electrical energy generated in two MFC reactors connected in series was also examined.

2. Methods

The cultures of *L. bulgaricus* and all the materials and equipment used to make the bacterial medium were obtained from the biochemistry laboratory of microbiology at Lembaga Ilmu Pengetahuan Indonesia (LIPI) Cibinong. For the MFC experiments, the materials and equipment were obtained from the laboratory of bioprocess at Faculty of Engineering, Universitas Indonesia. The voltage of the cell was measured using a digital multimeter (Sanwa Electric Instrument Co., Ltd., Japan) and the current was measured using an analog microampere (Yokogawa Electric Works., Ltd., Singapore).

Medium and bacterial inoculums. The bacteria were grown aerobically in a glucose yeast protein medium that consisted of glucose, yeast extract, beef extract, Tween 80, sodium acetate, and saline solution ($MgSO_4.7H_2O$; $MnSO_4.4H_2O$; $FeSO4.7H_2O$; NaCl). After sterilization using an autoclave, each culture of *L. bulgaricus* was added to the medium and incubated at 37 ^oC for 48 hours. The OD of the bacteria was measured using a spectrophotometer at a wavelength of 660 nm.

MFC configuration. The set up comprised a dual-chambered MFC composed of an anode and a cathode with a reactor volume of 100 mL in each chamber. In some experiments, a reactor volume of 500 mL was used, and the reactors were connected in series. The two compartments were connected with a proton exchange membrane (PEM; Nafion 117, Lyntech, USA) and joined tightly. The reactor scheme is shown in Figure 1.

The electrodes were carbon rods from used batteries, with an effective surface area of 13.67 cm^2. The electrodes were soaked in 1M HCl for one day, followed by immersion 1M NaOH for one day. The electrodes were completely immersed in the anode and cathode solutions and connected using a wire. Before use, the electrodes and the Nafion membrane were pretreated to remove any contaminants [8].

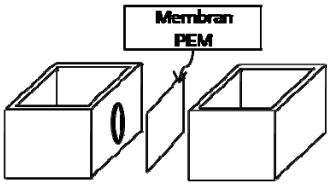

Figure 1. The Scheme of MFC Reactor

MFC experiments. The anode chamber was filled with *L. bulgaricus* inoculums, glucose, aquadest, and phosphate buffer (pH 7). Different values of bacterial OD were tested (0.5, 0.6, and 0.7). The cathode chamber was filled with potassium ferricyanide and a phosphate buffer (pH 7). In some experiments, potassium ferricyanide was replaced with potassium permanganate to investigate the effect of the electrolyte solution on the power generation. The MFC was operated forthree different durations, 3, 30, and 100 hours.

Analysis and calculation. Data on the currents and the voltages were obtained from the MFC experiments. The power density (mW/m^2) was defined as the power generated per unit area of the electrode. It was calculated using the following equation:

$$Power\ density\left(\frac{mW}{m^2}\right)=\frac{1\times V}{A} \quad (1)$$

where I (mA) is the current, V (V) is the voltage, and A (m^2) is the surface area of the electrode.

3. Results and Discussion

Effect of OD on electrical energy. The MFCs were tested at three different values of bacterial OD (0.5, 0.6, and 0.7) to obtain the optimum OD. As shown in Figure 2a and b, the highest OD value produced the smallest current and voltage. In other words, the greater the value of OD, the lower currents and the voltages.

The bacterial cell mass increased in accordance with the increase in the bacterial OD. Increasing the level of glucose did not result in an increase in the bacterial cell mass and the level of glucose in the anode chamber was rapidly depleted. The low level of glucose resulted in a decrease in the ability of *L. bulgaricus* to transfer the electrons from the bacterial cells to the electrodes [9]. This could explain why the maximum electrical energy was obtained at the low value of OD.

As shown in Figure 2c the highest power density (39.04 mW/m^2) was also generated by the MFC with an OD value of 0.5. At an OD of 0.6 and 0.7 the power density rapidly decreased compared to that at an OD of 0.5. As noted earlier, the magnitude of the OD value influences the bacterial cell mass. The level of glucose was depleted more rapidly when the bacterial cell mass increased. This resulted in a reduction in the number of protons and electrons transferred to the electrodes. Therefore, the power density value increased as bacterial OD decreased, the power density value was getting faster to decrease.

Effect of operation time on electrical energy. The MFCs were operated for 3, 30, and 100 hours. At the 3 and 30 hours operational times, the amount of electrical

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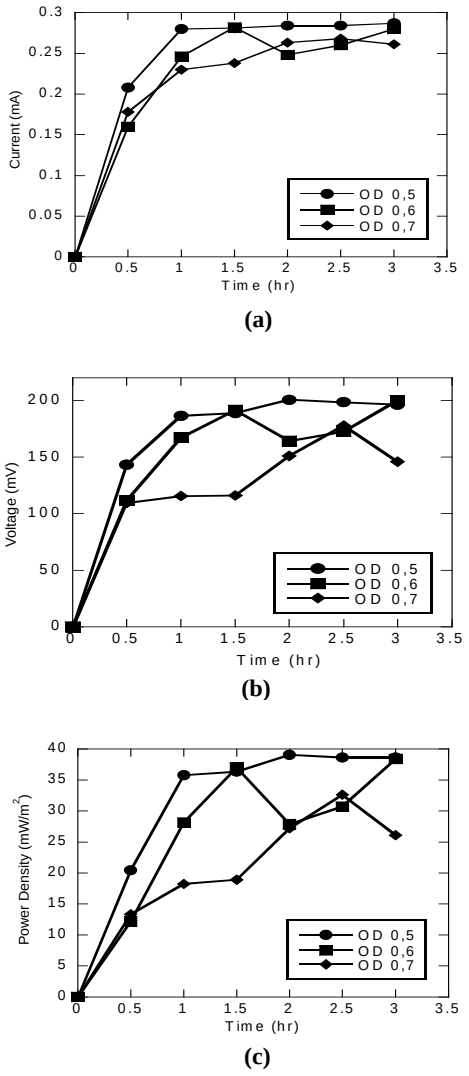

Figure 2. (a) Current, (b) Voltage, and (c) Power Density at Different Optical Density

energy generated continued to increase until the end of the observation period. The increase is related to the high level of glucose which enabled the rate of bacterial metabolism to remain high. As shown in Figure 3 the highest current, voltage and power density were achieved at an operational time of 100 hours.

Figure 4a shows that the current and the voltage increased sharply until it reached its maximum value after 38 hours of operation. They then remained relatively stable before starting to decrease at the 90th hour of operation. As shown in Figure 4b, the power density showed a similar trajectory.

The increase in the current, the voltage and the power density during the first 38 hours of operation is likely due to the breakdown of complex organic matter by bacteria [10]. Additionally, the bacteria were in the exponential phase with the cells actively metabolizing. In addition, the level of glucose was still high at this point enabling a high rate of electron transfer. After reaching the maximum value, the electrical energy produced stabilized. This was probably due to the bacteria entering the stationary phase. In the stationary phase, the number of living cells is equal to the number of dead cells.

By the end of the experiment, the electrical energy had decreased due to bacterial cell death and the depletion of the glucose reserves over time. Hydrogen produced as by-product of bacterial metabolism also led to a decrease in the electrical energy produced [4]. The amount of energy generated was further reduced by a biofilm layer on the proton exchange membrane and the electrodes [8], and a decrease in the conductivity of the solution due to the reduced concentration of phosphate buffer [2].

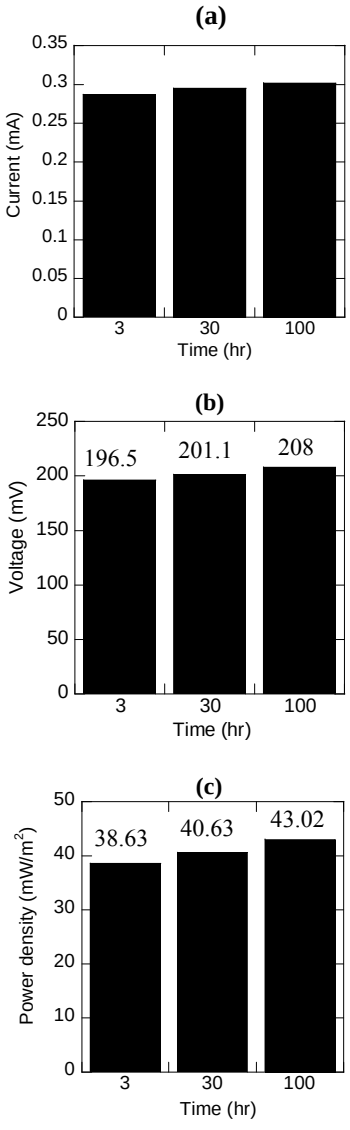

Figure 3. (a) Current, (b) Voltage, and (c) Power Density at Different Time Operation

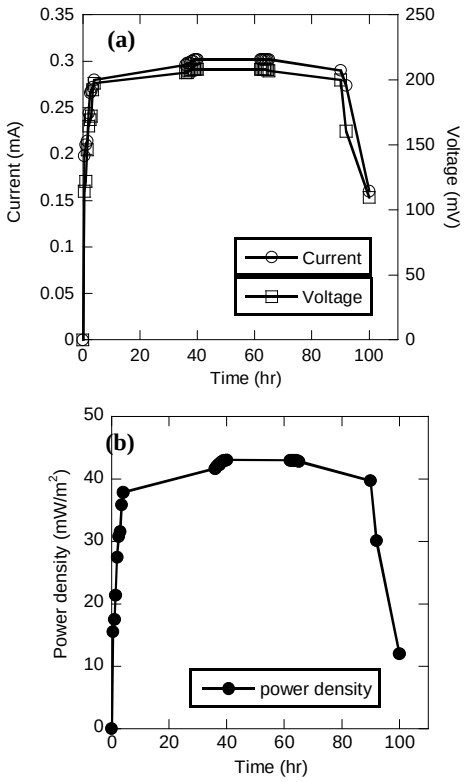

Figure 4. (a) Current and Voltage (b) Power Density at Time Operation of 100 Hours

Compared with the maximum current reported by Trinh *et al*. [4] who used *G. sulfurreducens* in a dual-chambered MFC, the maximum current produced in the current study was 59% greater. The maximum value also remained stable for up to 50 hours, whereas in Trinh *et al*.'s study, it remained stable only up to 40 hours. Moreover, the maximum current in the current study was achieved faster (at the 38th hour) than in Trinh *et al*.'s study (at the 80th hour). The maximum voltage also occurred earlier (at the 38th hour), in the present work than in the study by Guerrero *et al*. [10].

Effect of Reactor Volume on Electrical Energy. Two-chamber MFCs were tested using two reactors with different volumes, namely 100 and 500 mL. After 100 hours, the 500 mL reactor produced more electrical energy than the 100 mL reactor. The current increased by 49% (0.45 mA), and the voltage increased by 96.15% (408 mV). The power density increasedthree-fold (125.72 mW/m^2).

As shown in Figure 5a, the current and the voltage in the 100 mL reactor tended to remain stable during the first three hours. In contrast, the current and the voltage in the 500 mL reactor continued to rise until the maximum value was reached. They then started to show a relatively stable value at the 30th hour. The power density showed a similar behavior to the current and voltage behavior in the 500 mL reactor (Fig. 5b). As the 500 mL reactor contained a greater amount of glucose, the additional of glucose could increase the carbon source for bacteria, thereby allowing the metabolism process to last longer. Therefore, the 500 mL reactor was capable of generating higher electrical energy because the MFC power efficiency depends on the metabolism process [11].

In addition, the increased electrical energy was associated with the increase in the area of the proton exchange membrane in the 500 mL reactor. The greater membrane area allows a larger number of protons to migrate through the membrane.

Using a single-chamber reactor volume of 12.6 and 50 cm^3, Lorenzo *et al*. [12] obtained a similar conclusion to this study (i.e., a higher current is generated in a reactor with larger volume, and a smaller sized reactor reaches a stable value of electrical energy more quickly). This finding may be explained by the increased metabolic activity and the resulting high level of electron donors available in larger reactors [12-13]. The current produced by Lorenzo *et al*. was 44% smaller than in the current study because the reactor volume in this study was 40 times larger. The findings of the current study are also compatible with those of Qian *et al*. [13] who

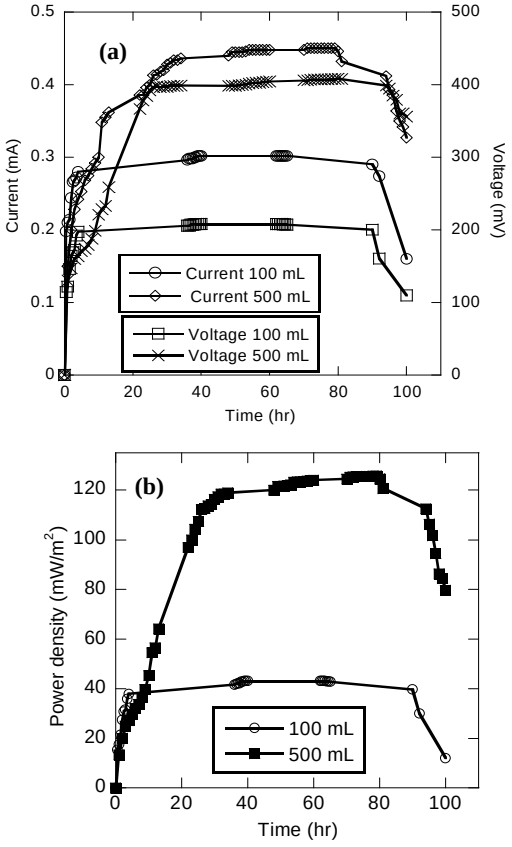

Figure 5. (a) Current and Voltage (b) Power Density Generated at Reactor Volume of 100 and 500 mL

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also found that a larger volume reactor was associated with more electrical energy.

Effect of type of electrolyte solution on electrical energy. In this experiment, potassium ferricyanide was replaced with potassium permanganate to investigate the effect of the type of electrolyte solution on the electrical energy. *L. bulgaricus* with a bacterial OD value of 0.5 was added to the 500 mL reactor. After 100 hours of observation, the maximum electrical energy generated was 0.536 mA, the voltage was 457 mV, and the power density was 167.7 mW/m^2.

As shown in Figure 6a, the maximum current and the voltage in the MFC with the potassium permanganate as the electrolyte solution showed an increase of 19% and 12% respectively. The increase was due to the high redox potential of the permanganate compared with that of ferricyanide, as seen in Eq. 2-3.

$$MnO_4^- + 4H^+ + 3e^- \rightarrow MnO_2 + 2H_2O \; E^0 = 1.70 \text{ V} \quad (2)$$

$$[Fe(CN)]^{3-} + e^- + [Fe(CN)]^4 \; E^0 = 0.36 \text{ V} \quad (3)$$

The anode potential is generally determined by several factors, such as the substrate conversion rate and the electrons transfer rate from the microorganisms to the anode. The cathode potential depends on the types of cathodic electron acceptor used. Assuming the redox potential of NAD^+/NADH in the anode is constant (-0.32 V), the voltage of the cell would be dependent only on the performance of the cathode. As permanganate has a high potential redox, there would be a greater potential difference between the anode and the cathode. As a result the electrical energy produced would increase [14].

In this experiment, the highest power density was obtained when potassium permanganate was used as the electrolyte solution, with an increase of 33.5% compared to potassium ferricyanide (Fig. 6b).

Similar results were obtained by Guerrero-Rangel [15] who compared the use of potassium permanganate, potassium ferricyanide, and potassium dichromate, with the highest electrical energy generated when potassium permanganate was used as the electrolyte solution.

Effect of MFC reactors in series on electrical energy. In this experiment, two reactors (100 mL and 500 mL) were combined and connected in series to examine the effect of the configuration of the reactor on the electrical energy produced. The MFC experiments were conducted using potassium permanganate as an electrolyte solution and a bacterial OD of 0.5. After 100 hours of observation, reactor connected in series produced a maximum voltage of 685.5 mV and a maximum power density of 201.8 mW/m^2.

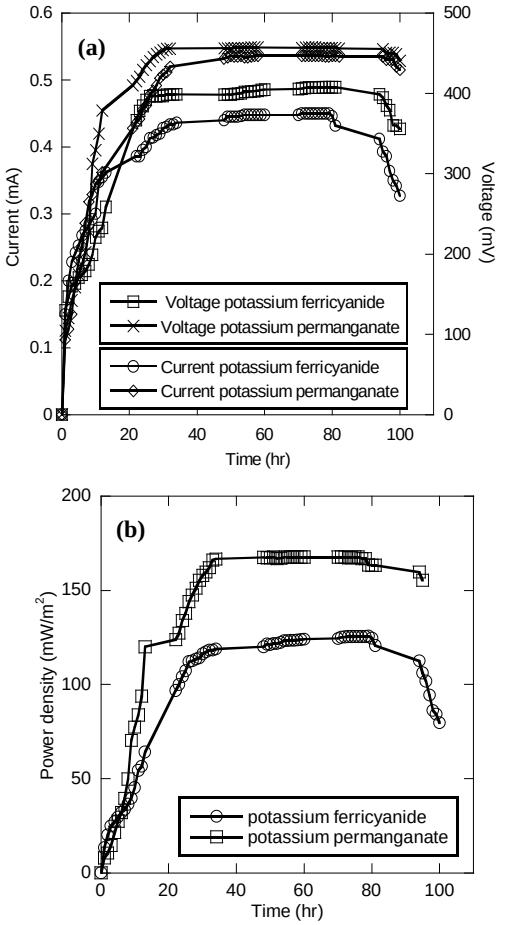

Figure 6. (a) Current and Voltage (b) Power Density at Different Type of Electrolyte Solutions

As shown in Figure 7a, the MFC reactor connected in series produced an increased voltage and a lower current compared with the single reactor volume of 500 mL. Overall, the reactor connected in series produced the highest power density, with an increase of 20.3%, as shown in Figure 7b.

The 50% increase in the voltage occurred because the total voltage in the connected series is the sum of each voltage from the power supply, which was the MFC reactor. Therefore, the reactor connected in series increased the voltage generated.

In contrast to the voltage, the current was decreased by 19.8% when the reactor was connected in series. The lower current was caused by the total internal resistance (R_{in}), which is the sum of the resistance in each MFC reactor. As the total resistance increased in the series connection, lower currents were generated [16].

In this experiment, the decrease in the current was not significant when compared to the increase of the voltage. Therefore, the overall power density increased in the MFC reactor connected in series. As noted by Momoh,

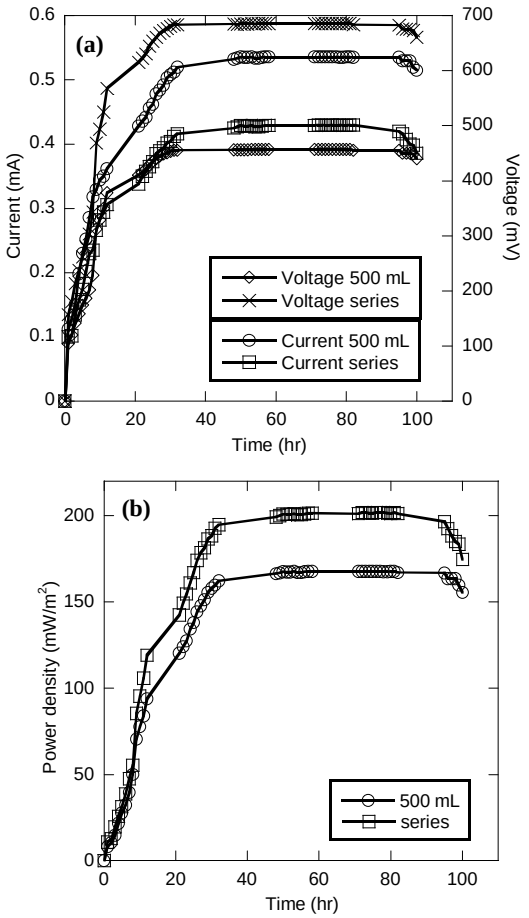

Figure 7. a) Current and Voltage (b) Power Density Generated at Single and Series-connected Reactor

this could increase the open circuit voltage value, thereby triggering an increase in the power density value [16].

4. Conclusions

The *L. bulgaricus* MFC system produced quantifiable amounts of electricity. The performance of the MFC system was affected by several factors (i.e., the OD, operating time, the reactor volume, the type of electrolyte solution, and the configuration of the reactor).

The optimum OD was 0.5, which can produced a current of 0.287 mA, a voltage of 200.7 mV, and a power density of 39.04mW/m^2. The maximum electrical energy was produced after 38 hours of operation, generating a current of 0.302 mA, a voltage of 208 mV, and a power density of 43.02 mW/m^2. Increasing the reactor volume to 500 mL increased the electrical energy. The current increased by 49%, the voltage increased by 96.15%, and the power density increased three-fold due to the increase in the glucose volume and the area of the proton exchange membrane in the 500 mL reactor. There was a greater rise in the electrical energy generated when potassium permanganate was used as the electrolyte solution than when potassium ferricyanide was used because the former has a higher redox potential. The maximum value of the current, the voltage, and the power density increased by 19%, 12% and 33.5%, respectively. Connecting the reactors in series led to an increase in the voltage and the power density of 50% and 20.3% respectively. However, due to the high internal resistance of the double dual-chamber reactor in the series, the maximum current decreased by 18.5%.

Acknowledgments

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