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Optimization of the electrical signal generation of a microbial fuel cell for sensor applications

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Abstract:

In previous studies, a microbial fuel cell (MFC) was developed as a potential sensor that detects iron in water. However, to realize such an application in practice, the electrical signal generation of the MFC must be improved. Therefore, in this study, we investigated several measures to optimize the electrical signals of the MFC including (i) changing the anode spacing, (ii) testing different oxygen supply rates, (iii) testing different external resistances, and (iv) testing a new electrode material. An anode spacing of 2 cm was found to be optimal as the MFC generated a current that was at least 2-fold higher than any other anode spacing investigated. To limit oxygen diffusion from the cathode to the anode, an optimal cathode air flow rate of 1.8 ml min^{-1} was found, which corresponds to an oxygen supply rate of 0.286 mg min^{-1}. By a polarization experiment, a 60-Ω external resistance ensured the most stable MFC-generated current , which is compulsory for the use of the device as a biosensor. Finally, activated carbon was shown to be an excellent material to improve electrical signal generation by 2-fold in comparison with graphite felt and graphite granules. These reported results will be the basis of further development of the MFC toward a practical biosensor.

Keywords: **anode spacing, electrode material, external resistance, microbial fuel cell biosensor, oxygen supply rate.**

Classification number: **3.5**

Introduction

Microbial fuel cells (MFCs) are bioelectrochemical systems that generate electricity throughthe electrochemical activity of microorganisms that harvest electrons by oxidizing substrates at the anode [1]. Due to this unique property, MFCs offer a variety of potential applications. These include the use of MFCs as sensors to analyse or monitor pollutants such as organic content or metals [2-5]. Particularly, Nguyen, et al. (2015) [4] successfully developed an MFC that can be potentially applied to detect iron and manganese in water samples. Reusability, long lifetime, and simple handling are some advantages of an MFC system [6]. However, to realize such a potential application in practice, the stability and sensitivity of the electrical signal generated by the MFC need to be improved [6]. In order to achieve this objective, the following factors influencing the performance of MFCs should be addressed [7, 8].

The performance of MFCs can be affected by operational parameters such as temperature, pH, dissolved oxygen concentration, and electrolyte (or buffer) strength [7, 9]. The power generation of MFCs may not reach their theoretical maximumdue to ohmic, activation, and concentration losses that cause overpotentials. Some proposed approaches to reduce these losses include (i) optimization of the reactor configuration, such as adjusting the electrode spacing, (ii) use of a highly proton-selective membrane, (iii) increasing the electrode surface area, and/or (iv) improving the activity of the catalysts at both electrodes [10]. Therefore, to improve the performance of the previously-developed MFC for sensor applications [4], in this study we attempt to (i) discover a better performing electrode material to

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minimize the internal resistance of the MFC, (ii) modify the anodic electrode spacing to determine a design that better supports the electrochemical activity of acting bacteria, and (iii) reduce losses during the electron transfer process (from the electron donor at the anode to the electron acceptor at the cathode) via optimization of the cathode oxygen supply rate and the external resistance using polarization analysis.

Materials and methods

Reactor setup

The MFC reactors used in this study were fabricated based on the design of the lithotrophic iron-oxidizing MFC (LIO-MFC) previously developed in [4]. In brief, each reactor consisted of two large poly-acrylic frames (12 cm×12 cm×2 cm) and two small poly-acrylic rectangle-holed subframes of anode and cathode compartments (8 cm×8 cm×X cm), with X being 1.5 cm unless specified as the spacing value to be tested in the respective experiment. Each rectangle hole on each subframe had the dimension of 5 cm×5 cm and thus the dimension of each compartment was 5 cm×5 cm×X cm. Graphite granules (3-5 mm in diameter) were used as the default electrode material and thus loaded into each compartment until it was filled. These were replaced with activated carbon granules in an experiment specified below. These granules were loaded in a manner to ensure that they were packed well enough to ensure good contact with each other and with the graphite rod (5 mm in diameter) to collect the electrical current [4]. Epoxy glue was used to seal the gaps between the rod and the frame to ensure that the compartment was completely closed. Also, for this purpose, rubber gaskets were sandwiched between the poly-acrylic parts during reactor assembly. Two compartments of each reactor were separated by a 6 cm×6 cm Nafion 117 membrane (Du Pont, USA). The rest of the reactor assembly process was the same as described in [4]. A default external resistance of 10 Ω was used unless otherwise stated in an experiment.

The anolyte or the catholyte was conveyed in and out of their respective chambers in each reactor through PVC pipes sealed to 2 holes (5 mm in diameter) that were created on the large frame of each compartment. The sterilized modified M9 medium (0.44 g KH_2PO_4 l^{-1}, 0.34 g K_2HPO_4 l^{-1}, 0.5 g NaCl l^{-1}, 0.2 g $MgSO_4.7H_2O$ l^{-1}, 0.0146 g $CaCl_2$ l^{-1}, pH 7) was contained in a medium bottle and was passed through a drip chamber before being supplied to the anode chamber via the anode influent pipe inserted with a three-way connector [11].

Operation of the MFCs [4]

After assembly, the MFC reactors were operated as in previous studies [4, 12]. Specifically, in batch mode, at room temperature (25±3°C), with $FeCl_2$ as the source of ferrous ions mixed in the modified M9 medium through the three-way connector on the anode influent pipe. The modified M9 medium was purged with nitrogen (Messer, Vietnam) for 30-60 minbefore being supplied to the anode to minimize the amount of oxygen, which may compete with the anode to accept electrons. The final concentration of Fe^{2+} in the anolyte was achieved by a careful calculation of the volume and the concentration of the supplied $FeCl_2$ solution. In each batch, half of the anolyte (approx. 10 ml) was replaced. A $NaHCO_3$ solution was also supplied as the carbon source for the anode microorganisms such that its final concentration in the anolyte was 2 g l^{-1} [11]. At the cathode of each MFC reactor, only a buffer solution without any catalyst (0.44 g KH_2PO_4 l^{-1}, 0.34 g K_2HPO_4 l^{-1}, 0.5 g NaCl l^{-1}) was supplied. Any remaining catholyte was completely replaced with freshly-made catholyteat the beginning of each batch. The cathode compartment was aerated through the cathode influent pipe with an air pump (model SL-2800, Silver Lake, China) to provide the final electron acceptor, which is oxygen. The pump was manipulated so that the rate of aeration was slightly above 50 ml min^{-1} to ensure that the cathode solution was air-saturated [13] but did not evaporate quickly. In this study, the manner of oxygen supply was altered in some experiments, which is described later.

A batch run was startedwhen the anolyte was replaced, and the run was finished when the current dropped down to its baseline. Thus,such a batch usually lasted for 2 h. In experiments, an interval of 1 h was allowed in between every 2 consecutive batches. The MFC reactors were left on standby during the night. The operational scheme described above did not affect theperformance stability of the MFC reactors.

Enrichment of iron oxidizing bacteria in the MFCs

For the enrichment of iron-oxidizing bacteria in the MFCs, two different microbial sources were used for inoculation: (i) well water samples, with a fawn colour typical for water contaminated with iron, taken from Hoai Duc and Hoang Mai (Hanoi, Vietnam) and (ii) soil and mud samples at a depth of 10 cm from the Trai Cau iron mine, Dong Hy (Thai Nguyen province, Vietnam). The two sources were mixed at a ratio of 1:1 to create an inoculum for the enrichment. The

enrichment process was the same as in previous studies [4, 12], with 20 mM being the concentration of Fe^{2+} supplied into the anode during the enrichment.

Testing activated carbon granules as a novel electrode material

Activated carbon granules (COCO AC Ltd., Vietnam) about 3-5 mm in diameter were tested as the electrode material in an MFC at both the anode and the cathode. Graphite granules were used in another MFC as the control. The graphite and activated carbon granules were washed several times with distilled water to remove impurities and were left to drain. After that, the granules were loaded into the electrode chambers of the MFCs. The installation of the MFCs with the granules was performed in the same way as described in the previous studies [4, 12]. At the same time, the MFCs in the experiment were enriched with electroactive bacteria from the same microbial sources. After the enrichment, the performance of the MFCs, in terms of electricity generation, were investigated and compared by using the methods described below.

Testing different cathode oxygen supply rates

This experiment was conducted to investigate and optimize the rate of oxygen supply to the cathode of the LIO-MFCs. In this experiment, instead of pumping air directly into the cathode compartment, we purged the catholyte separately with air in a flask at full speed by an air pump before supplying the aerated catholyte into the cathode compartment. In the same manner, the rate of oxygen supply to the cathode was adjusted with a speed control valve inserted into the cathode influent pipe that conveyed the catholyte from the flask to the cathode compartment. Two MFCs were operated with cathode flow rates ranging from the lowest speed of *ca.* 0.12 ml min^{-1} to a speed as high as 30 ml min^{-1}. Considering that the aerated catholyte in the separate flask was air-saturated, the oxygen supply rate corresponding to each flow rate can be calculated. During the experiment, the MFCs were fed with 5 mM of Fe^{2+} at the anode.

Testing varied external resistances and polarization analysis

In this experiment, we attempted to establish the polarization curve of the LIO-MFC by changing its external resistance and measuring the corresponding voltage and current. The external resistance values ranging from 5000 Ω to 10 Ω were tested accordingly. Throughout the test, the appropriate and adequate time for the current to stabilize at each resistance level was about 5 min. Thus, the MFC was operated with each resistance for about 5 min and then corresponding voltage and current were recorded. The average voltage of 5 measurements in 5 min was calculated and used for the calculation of other parameters such as current density, power density, etc.

Testing different anode spacings

To investigate the effect of the anode spacing on the performance of the MFCs, different thicknesses of the anode chamber were tested, including 1.5 cm (the default thickness used in the previous studies), 2 cm, and 2.5 cm. Three MFCs with anode spacings of 1.5 cm, 2 cm, and 2.5 cm were assembled and inoculated with the microbial sources for the enrichment of electroactive bacteria, as mentioned above, before their electricity generation performances were evaluated and compared.

Measurement and calculation of electrical parameters

A data acquisition system coupled with a multimeter (Keithley model 2700, Keithley Inc., USA) was used to automatically record the voltage between the anode and the cathode of each MFC. The recording interval was 1 min or 10 min depending on each experiment. The measurement and calculation of the following electrical parameters:current I (A), voltage U (V), power P (W), and resistance R (Ω) were carried out according to Logan, et al. (2006) [1] and Aelterman, et al. (2006) [14]. Unless otherwise stated, all the experiments in this study were repeated at least 3 times before the data were collected and analysed.

Results

Activated carbon - an electrode material suitable for sensors based on MFCs

Replacing the electrode material with activated carbon granules strikingly improved the generation of electrical current. The assembled LIO-MFC that was setup and operated with activated carbon granules produced a stable current of ca. 0.65 mA, which is more than 3-fold higher than that of the control with graphite granules (see Fig. 1). The increased current by the former was not intermittent but steady (Fig. 1). Polarization analyses also showed that the LIO-MFC with activated carbon granules produced about a 2-fold higher power density and a 4-fold higher current density compared to the control (see Fig. 2).

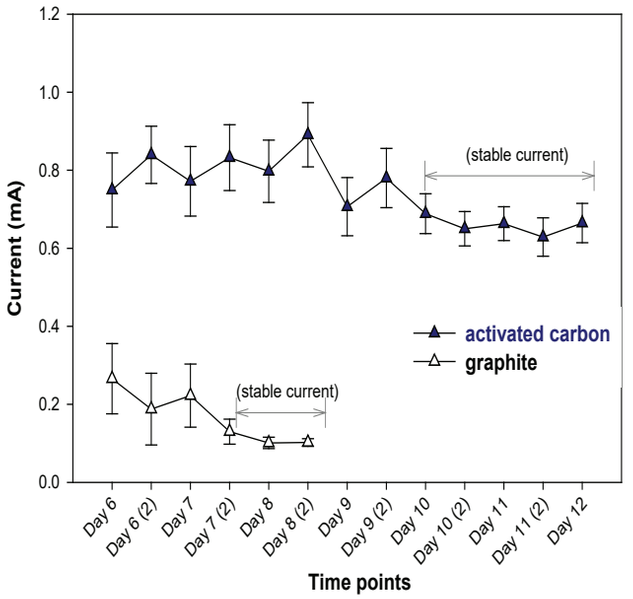

Fig. 1. Comparison of the electricity generation by the LIO-MFC operated with activated carbon granules as the electrode material (MFC 9) and the control operated with graphite granules as the electrode material (MFC 8). Both MFCs had an anode spacing of 1.5 cm and were operated at room temperature with an external resistance of 10 Ω and with directly-aerated cathodes.

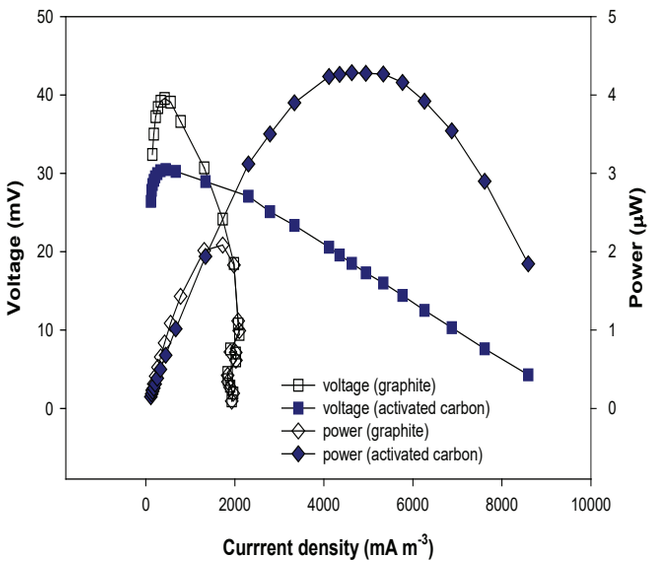

Fig. 2. The polarization curves performed on an MFC operated with activated carbon granules as the electrode material (filled symbols) and an MFC operated with graphite granules as the electrode material (unfilled symbols). Both MFCs had an anode spacing of 1.5 cm, and were operated at room temperature with an external resistance of 10 Ω, and with directly-aerated cathodes.

Activated carbon has been reported to have a larger surface area (thus larger contact area) and a higher catalytic activity for oxygen reduction when compared to graphite [15]. Increased surface area might reduce activation loss and diffusion loss and improve electron transfer [16]. It was reported that the catalytic activity for the oxygen reduction of activated carbon is over 3-fold higher than that of plain carbon (even better than that of graphite) and comparable to that of platinum [17]. Therefore, it is understandable that using activated carbon granules as the electrode material could improve the generation of electrical signals of our MFCs (the LIO-MFCs). Furthermore, our results indicate that this improvement can be stable in a long term. This point is also supported by Zhang, et al. (2011) [18], who reported that the material could stably perform for up to 1 year.

Determination of an appropriate oxygen supply scheme at the cathode of the MFCs to limit oxygen diffusion to the anode

Studies have shown that when oxygen is excessively supplied to the cathode, additional oxygen diffusion from the cathode to the anode can occur, which reduces electricity generation [9, 19]. It has also been reported that oxygen diffusion from the cathode chamber to the anode chamber can greatly affect the electron transfer and microbial community of the anode, therefore reducingthe generation of electricity [8, 20]. Therefore, our hypothesis is that our default mode of cathode aeration (as described above, at a rate of 200 l air h^{-1}) could lead to a rate of oxygen supply to the cathode that is too high, resulting in excessive dissolved oxygen levels and critical oxygen diffusion. Thus, we propose that the generation of electrical currents by the LIO-MFCs can be improved by a proper oxygen supply scheme at the cathode.

Therefore, various oxygen supply rates at the cathode were carefully tested by varying the air-saturated catholyte rates supplied to the cathode from 0.12 ml min^{-1} to 30 ml min^{-1}. Interestingly, the results (see Fig. 3) showed that the currents generated by the 2 MFCs in the experiment increased when the catholyte supply rate increased from 0.12 ml min^{-1} to 1.8 ml min^{-1} and clearly decreased when the catholyte supply rate was higher than 1.8 ml min^{-1}. At a catholyte flow rate of 1.8 ml min^{-1}, the currents generated by the 2 MFCs (MFC 6 and MFC 7) were 0.062 mA and 0.051 mA, respectively, which is almost double the currents generated at rates in the range of 11-30 ml min^{-1} (i.e.

excessive oxygen supply). This scheme of oxygen supply is much less intensive than our default direct aeration mode, even at high catholyte flow rates. From this we deduce that direct aeration mode is far from optimum and causes too much oxygen diffusion, as proposed in our hypothesis.

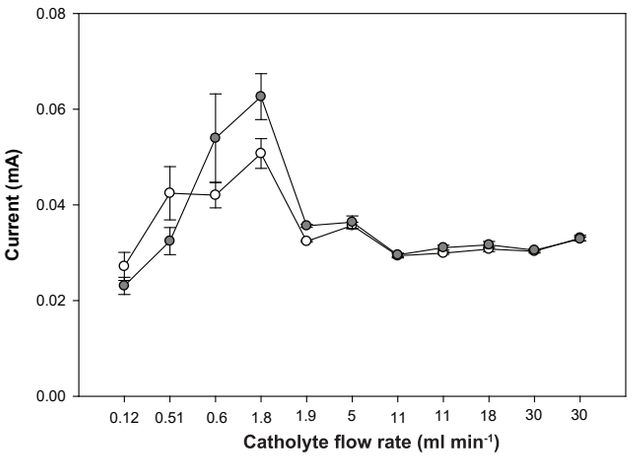

Fig. 3. The relationship between the air-saturated catholyte flow rate and the current generated by the LIO-MFC. Two MFCs were used as replicates. The MFCs both have an anode spacing of 1.5 cm, were operated at room temperature with graphite granules as the electrode material, and with an external resistance of 10 ohm.

As mentioned above, cathode-to-anode oxygen diffusion was discovered a long time ago, but to our knowledge no study has been conducted to determine a proper oxygen supply at the cathode to limit that diffusion and its consequence. In this study, we report for the first time, an optimal oxygen supply rate to the cathode, which is 1.8 ml air-saturated catholyte min^{-1} equal to 0.286 mg O_2 min^{-1}. Knowing this value will not only support the operation of MFCs in a way that minimizes the oxygen diffusion, but also help save energy for cathode aeration.

Determination of an optimal external resistance for a high and stable generation of the LIO-MFC

A polarization curve of a LIO-MFC operated with activated carbon granules as the electrode material was established by varying the external resistance in order to determine the condition at which the power density is maximum. The polarization curve (Fig. 2) showed that the power density of the MFC reached its maximum when the current density was in the range of 4200-4700 mA m^{-3}. Under these conditions, the external resistance was about 60-100 Ω (Fig. 4). In this range, the current is proportional to the voltage (Fig. 4), which indicates a stable performance of the system.

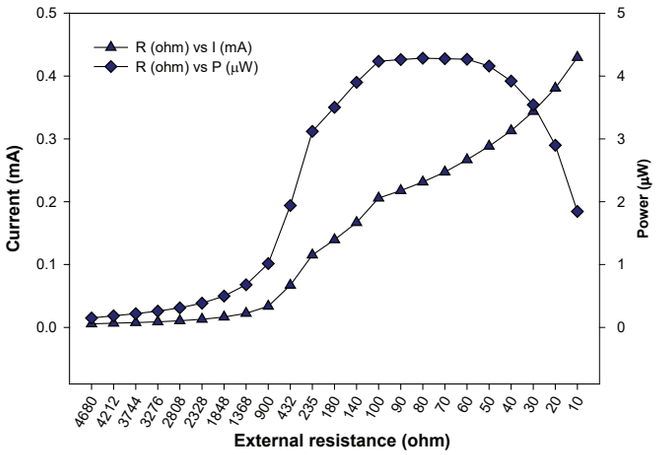

Fig. 4. The electricity generation of the LIO-MFC operated with activated carbon granules in response to changes in the external resistance.

The effect of external resistance on the performance of MFCs, in general, has been reported by a number of publications [9, 21] and the need to determine an optimal external resistance is evident [22]. It is believed that a proper match between the external resistance and internal resistance is required for a good performance of an MFC [22, 23]. While an external resistance of less than 500 Ω was suggested for use in certain types of BOD-sensing MFCs [9], a much lower external resistance (10.5 Ω) was suggested to improve and stabilize the performance of some other systems [24]. It is therefore plausible that there is a specific optimal external resistance for each individual system. In our study, an external resistance between 60 and 100 Ω appears to enable an optimal generation of electricity by the LIO-MFC.

Effect of anode spacing on the generation of electrical signals by the LIO-MFCs

As described above, 3 MFCs with different anode spacings (1.5 cm, 2 cm and 2.5 cm) were assembled and inoculated with the microbial sources for the enrichment of electroactive bacteria. The 3 MFCs began to generate electrical currents right after the first day of enrichment when operated with 20 mM Fe^{2+} at the anode. The currents gradually became stable 3 to 5 d after the inoculation. The average daily currents of the 3 MFCs were significantly different ($p<0.05$, Fig. 5). Among the 3 MFCs, the one operated with an anode spacing of 2 cm (MFC 7) showed the best performance with a generated current of around 0.3 mA. The current generated by the MFC operated with an anode spacing of 2.5 cm (MFC 6) was only around 0.25 mA and that of the MFC with 1.5 cm anode spacing (MFC 1)

was even lower at 0.15 mA. The results show that the anode spacing greatly affects the electrochemical processes inside the MFCs.

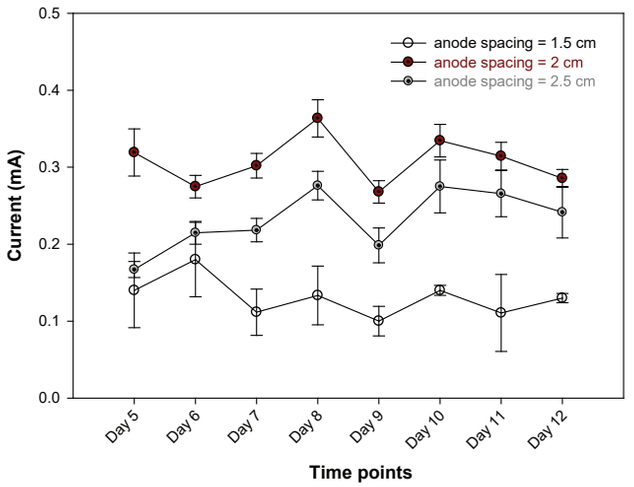

Fig. 5. The differences in electrical signals generated by 3 MFCs with different anode spacings: 1.5 cm (MFC 1) (the control), 2.0 cm (MFC 7), and 2.5 cm (MFC 6). The MFCs were all operated at room temperature with graphite granules as the electrode material, with an external resistance of 10 Ω, and with directly-aerated cathodes.

The currents of the 3 MFCs followed similar trends in time while the MFCs were operated under the same conditions, which indicates that the differences in the electrical current level are due to the differences in their anode spacings. It is interesting to note that increasing the anode spacing from 1.5 cm to 2 cm could significantly boost the current, but increasing 0.5 cm further led to a reduced current. The latter is the reason why we did not test further increased anode spacings. An increased anode volume may permit increased substrate supply and an increased surface area for the anode reaction, but this does not always mean that a larger anode volume generates a higher electrical current. Furthermore, a shorter electrode spacing is believed to reduce the internal resistance, resulting in better electron transfer and thus a higher power output [24, 25]. However, reducing the anode spacing to a certain level could lead to reduced electricity generation, possibly due to oxygen intrusion from the cathode to the anode [25]. Thus, it is always necessary to determine an optimal anode spacing for any MFC system. In our case, the anode spacing of 2 cm appears to be close to optimum. Our results also demonstrate that the current generated by the MFC with 2 cm anode spacing was more stable, which is a beneficial property for an MFC-based sensor.

Conclusions

In this study, several measures to improve the generation of electrical signals by a potential lithotrophic MFC-based sensor were found by combining the advantages of MFC performance optimization from previous studies. These include (i) increasing the anode spacing to an optimal value of 2 cm, (ii) replacing the electrode material with activated carbon granules, (iii) operating the MFC with an oxygen supply rate of 0.286 mg O_2 min^{-1} at the cathode, and (iv) operating the MFC with an external resistance in the range of 60-100 Ω. Upon those findings, further studies are required to realize the application potential of the improved system as a biosensor for environmental monitoring in practice.

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The authors declare that there is no conflict of interest regarding the publication of this article.

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