

## Biosynthesis of zinc oxide nanoparticles by using fruits extracts of *Ananas comosus* and its antibacterial activity

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### Abstract

Biosynthesis of metallic nanoparticles using plants, enzymes, and microorganism has been known as eco-friendly alternative compared to other conventional physical and chemical methods. Recently, the biological synthesis of nanoparticles has been a keen interest among researchers and scientists due to its simple technique, eco-friendliness, non-toxic and low in cost. Thus, in this current work, the synthesis of zinc oxide nanoparticles (ZnO-NPs) using reduction agent from fruit extracts of *Ananas Comosus* was reported. The biosynthesized ZnO-NPs were characterized using Field Emission Scanning Electron Microscope (FESEM) paired with Energy Dispersive X-ray analysis (EDX), UV-Vis absorption spectroscopy and X-ray diffraction (XRD). The average size of the nanoparticles was found to be in the range of 30-57nm. The antibacterial activity of ZnO-NPs was performed via agar diffusion method against pathogenic organisms. It was observed that the biosynthesized ZnO-NPs in the process has the efficient antibacterial performance against *Escherichia coli* (E-coli). In conclusion, the green synthesized ZnO-NPs using the fruit extract of *Ananas Comosus* was successfully conducted and interestingly it has also been proved to exhibit antibacterial effect against *E. coli* strains.

**Keywords:** Green synthesized ZnO, *Ananas comosus*, fruit extract, nanoparticles, antibacterial activity

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## INTRODUCTION

Researchers nowadays have keen interest towards zinc oxides (ZnO) especially in nanostructure scales due to their wide applications and unique properties. The widely use of ZnO and tremendous interest recently, especially in the semiconductor and photocatalytic application, are resulted from a direct wide gap (3.37 eV, 387 nm), deep violet/borderline ultraviolet (UV) and a large exciton-binding energy (60 meV) (B. Kumar et al. 2014). ZnO is a highly preferred material as it ables to act as multitasking metal oxide, with a vast list of attractive properties for various applications. Due to its exceptional optical and electrical properties (Könenkamp et al. 2002), it is regarded as a potential material to be used in optoelectronic applications which is effective in the visible and near ultraviolet spectral regions. Zinc oxide nanoparticles (ZnO-NPs) are widely used in many industrial areas such as solar cells (R. Vittala 2017), UV light-emitting devices (Y.-J. Lu et al. 2017), gas sensors (Galstyan et al. 2015), photocatalysts (Chen et al. 2017), pharmaceutical (Karimi-Maleh et al. 2014) and cosmetic industries (P. J. Lu et al. 2015).

Recently, ZnO has turned out to be one of the most crucial and multifunctional semiconductor materials which extensively applied as

photo-catalyst and antibacterial materials. Balta *et al.* (2012) in their study reported that the utilization of ZnO-NPs did not create and increase toxicity level since the surface area and size distribution of the particles were too small, making the effect was too far from toxicity level. (Balta et al. 2012).

In fact, ZnO-NPs are safer and less toxic compared with other metal oxide nanoparticles, therefore it has been increasingly used in applications such as food industries for processing and packaging of vegetables and meats (Raj and Jayalakshmy 2015). There are various methods that have recently been used for the synthesis of ZnO-NPs, such as sol gel method, direct precipitation, thermal decomposition, sonochemical method, microwave irradiation, solvothermal method, reverse micelles, hydrothermal and homogeneous precipitation (Arora et al. 2014). However, the application of chemical and physical methods in production of ZnO-NPs is yet costly and hazardous.

Recently, there is growing need to environmental friendly nanoparticles synthesis method known as biological synthesis, where the process of synthesizing nanoparticles by using plants, enzymes, alga and microorganisms have been proposed. This approach offers an eco-friendly alternative in synthesizing route compared to the physical and chemical methods, along with better effect and

performance (Azizi, Ahmad, and Mohamad 2013). As for example, the ZnO-NPs were successfully synthesized by using plants of *Lycopersicon esculentum* extract (Sutradhar and Saha 2017), *Allium sativum*, *Rosmarinus officinalis* and *Ocimum basilicum* extracts (Stan et al. 2016), *Trifolium pratense* flower extract (Dobručka and Długaszewska 2015) and Root Extract of *Zingiber officinale* (Raj and Jayalakshmy 2015). Further investigation on this approach also was conducted by Zheng et al. (2015) and Azizi et al. (2014), that reported on the synthesis of ZnO-NPs using *Corymbia citriodora* leaf extract and marine macroalga *Sargassum muticum* aqueous.

Utilization of ZnO-NPs is beneficial due to their ability to inhibit the growth of pathogenic microbes in minute concentration (Applerot et al., 2009). Based on the previous study, it was reported that ZnO exhibited significant growth in inhibition of a broad spectrum of bacteria. The antibacterial activity of ZnO-NPs was reported against clinical isolates: *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Jeeva et al., 2012). In the previous work, Prasad and Krishna (2016) attempted to synthesize ZnO-NPs using the *Boswellia ovalifoliolata* stem bark extract. The results showed that ZnO-NPs exhibited good antibacterial activity against Gram positive bacteria and Gram negative bacteria.

In this study, pineapple (*Ananas comosus*) was used as the reduction agent to synthesize the ZnO-NPs. *Ananas Comosus* is a tropical plant which broadly cultivated for its unique feature of yellow flesh and sweet juice. Now, pineapple is the world's third most essential cultivated tropical fruit after bananas and citrus. The suitable place for pineapple to grow is in frost-free areas around the world. Nutritionally, pineapple is an excellent source of vitamins C, A and also potassium. Recent studies proved that vitamin C presented in plants has potential to be used for the synthesis of zinc oxide and silver nanoparticles (Sutradhar and Saha 2017, Shrikant R. et. al. 2011).

Therefore, the aim of this current study was to investigate the properties of *Ananas comosus* as the reduction agent for the biosynthesis of ZnO-NPs. Further investigation on the effect of antibacterial properties of the biosynthesis ZnO-NPs was also carried out.

## EXPERIMENTAL

### Materials

The *Ananas Comosus* (pineapple) fruits were collected from the local source in Parit Raja, Johor, Malaysia. Zinc nitrate was purchased from Sigma Aldrich and distilled water was used throughout the experiments.

Bacterial strains used for the antibacterial test of ZnO nanoparticles was *Escherichia coli* (*E. coli*) ATCC 25922.

### Preparation of pineapple fruit extract

The fresh *Ananas Comosus* (pineapple) fruits were collected from the local market and the skin was cut thoroughly. Then, it was washed with double distilled water and cut into small pieces subsequently. After that, the small pieces of pineapple were squeezed to get the juice. The juice was then dissolved in distilled water with stirring time of 10 minutes at 40°C and filtered using a Whatman filter paper (Sutradhar and Saha 2017). The filtrate was collected and kept below 10°C for further use. A step flow chart of the juice preparation was as in Fig. 1.

### Bio-synthesis of ZnO-NPs

The precursor for the ZnO-NPs in this synthesis was zinc nitrate. The zinc nitrate and pineapple extract were mixed in the ratio of 1:3 and stirred at 240°C for 5 minutes until brownish-black precipitation was produced. The solution was then centrifuged at 10000rpm for 7 minutes. The precipitates were collected and then dried in oven at 40-100°C. All the precipitates were collected in glass container to be further characterized.

### Preparation of pineapple fruit extract :

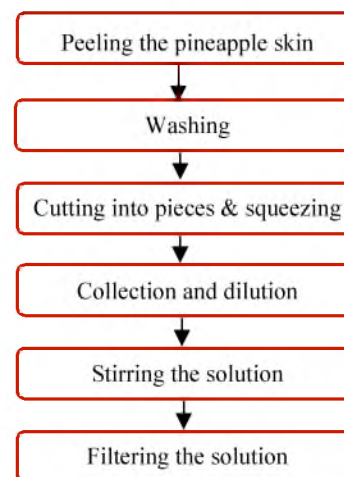


Fig.1. Preparation of pineapple fruit extract.

### Characterization of ZnO-NPs

#### X-ray diffraction (XRD)

The X-ray diffraction (XRD) measurement of biosynthesized ZnO-NPs was carried out using an X-ray diffractometer with Cu K $\alpha$  radiation ( $\lambda = 0.154$  nm, D/max-rB 12 kW Rigaku) which operated at 30 mA and 40 kV from 10° and 80° with a step increment rate of 0.05°/min. The estimation of the particle size was performed by using Scherrer's formula (1).

The Scherrer's formula could be used to calculate the crystalline size of particle

$$D = K \lambda / \beta \cos \theta \quad (1)$$

where,

$D$  is the crystalline size of the particles,  
 $\lambda$  is the wavelength of the X-ray used,  
 $K$  is the shape factor,  
 $\beta$  is the full line width at the half-maximum elevation of the main intensity peak, and  
 $\theta$  is the Bragg angle.

#### Field emission scanning electron microscope (FESEM)

The shape and size of the synthesized ZnO-NPs were examined by using Field emission scanning electron microscope (FESEM). The samples were placed on an adhesive carbon tape supported on copper stubs and coated with 10 nm thick gold using JEOL JFC-1600 auto fine coater before measurement (Karunakaran, Rajeswari, and Gomathisankar 2011).

#### Energy dispersive X-ray diffraction (EDX)

The energy dispersive X-ray diffraction (EDX) which attached with FESEM was used to obtain the elemental analysis with acceleration voltage of 15.0 kV and working distance of 11.6 mm and 15.0 mm for FESEM and EDX, respectively. Copper tape was used as tape detector.

#### UV-Vis Absorbance for ZnO-NPs

As for UV-Vis spectra analysis, the maximum absorbance was measured by using UV-Vis Spectrophotometer Perkin Elmer model G10S UV-Vis with wavelength in the range of 280 – 800 nm.

#### Antibacterial activity of the biosynthesized ZnO nanoparticles

The agar diffusion method was used to test the ZnO-NPs for their antibacterial activity against strains (Stan et al. 2016). The *E. coli* bacteria were grown in nutrient broth to prepare stock solutions of 100 µg/mL. Later, 100 µL of bacteria inoculums was spreaded over the

plates containing sterilized nutrient agar. Paper filter disks (6 mm) were impregnated with 20  $\mu$ L of stock solution. The plates were maintained at room temperature for 30 min to allow the diffusion of the solutions. The inhibition zone around the disk was measured after incubation at 37 °C for 24 h (Karunakaran, Rajeswari, and Gomathisankar 2011).

## RESULTS AND DISCUSSION

### XRD patterns

Fig. 2 shows the XRD pattern of the biosynthesized ZnO-NPs. The distinct diffraction peaks at  $2\theta = 31.717^\circ$ ,  $34.484^\circ$ ,  $36.245^\circ$ ,  $47.585^\circ$ ,  $56.705^\circ$ ,  $62.826^\circ$ ,  $66.580^\circ$ ,  $68.025^\circ$  and  $69.039^\circ$  were

assigned to (100), (002), (101), (102), (110), (103), (200), (112) and (201) planes, respectively. All the relative peaks of the biosynthesized ZnO-NPs were matched with the Joint Committee on Powder Diffraction Standard (JCPDS) card number 00-065-0725 in terms of intensities and positions. All the peaks recorded in Fig. 2 were well indexed to the hexagonal phase of ZnO and the nanopowder was shown to be highly crystallized owing to the narrow and sharp peaks. The high intensity at the peak of (101) was as an indicative of anisotropic growth and thus, implying on a preferred orientation of the crystallites.

The crystallite size of the particles that calculated using this equation was found to be 12.2 nm.

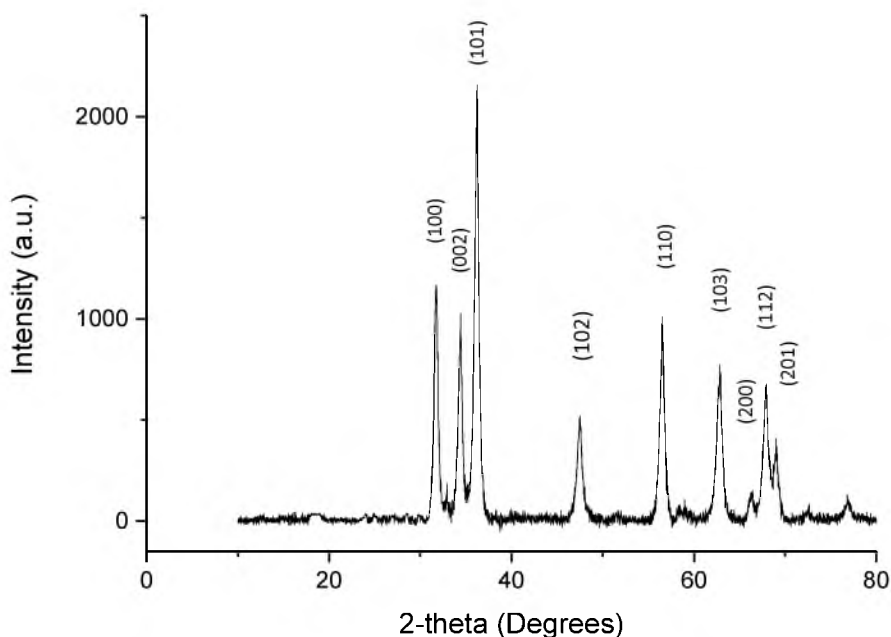


Fig.2. XRD pattern of biosynthesized ZnO nanoparticles.

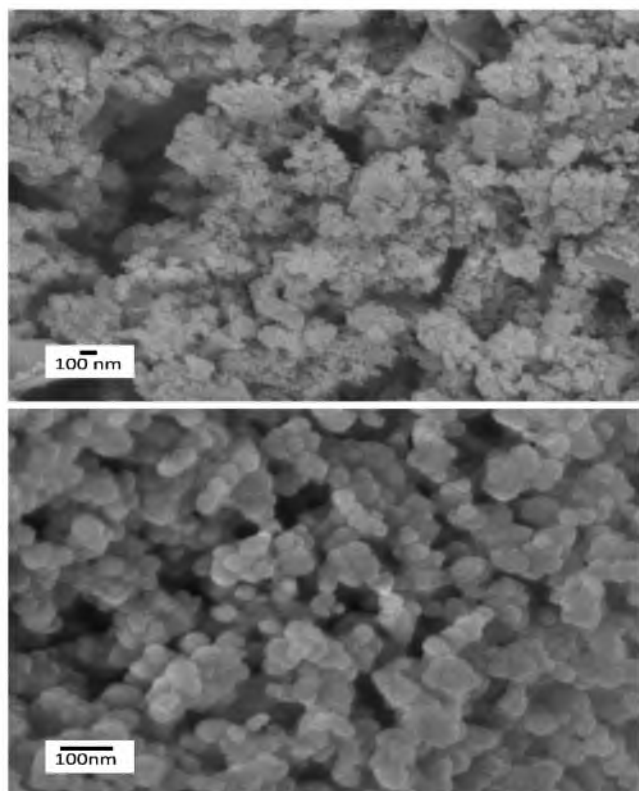


Fig. 3 FESEM images of ZnO-NPs.

### FESEM analysis

FESEM images of biosynthesized ZnO-NPs were shown in Fig. 3. It could be observed from Fig. 3 that the synthesized nanoparticles formed were agglomerated with the hexagonal structures and the diameter size of the particles was ranged from 30 to 57nm. According to Raj and Jayalakshmy (2015), the particle size of the synthesized ZnO nanoparticles was in the range of 30-50nm which was comparable to the results obtained in the present study. The spot numbered 1, 2, 3, 4 in the Fig.3 was indicated for the points in which the measurement was made.

### EDX analysis

The elemental analysis of the biosynthesized ZnO-NPs was carried out by using EDX to get further confirmation on the presence of ZnO-NPs in the sample. The EDX spectrum of biosynthesized ZnO-NPs was shown in Fig. 4. The EDX result showed that the peaks were exhibited between 1 kV and 6 kV. The peaks were directly related to zinc and oxide that presented in the tested material. The results also presented that the biosynthesized ZnO-NPs from fruit extract of *Ananas Comosus* was made of high purity zinc nanoparticles. According to S. S. Kumar et al. (2013), the energy dispersive spectra of the samples obtained from the FESEM-EDX analysis showed that the sample prepared by the above route has pure ZnO phases which was similar to the results obtained in the present study. Then, the elemental composition of ZnO-NPs sample was summarized in Table 1. The presence of oxygen and zinc elements in the nanoparticles was confirmed by the EDX results. The spectrum in Fig. 3 showed that ZnO-NPs were consisted of O and Zn elements with the percentages of 31.62% and 68.38% that given in Table 1 respectively, thus proving that the produced ZnO-NPs were in highest

purified form and also were in agreement with the earlier studies (Raj and Jayalakshmy 2015). It was shown that the significant peaks obtained for the ZnO sample were similar to the peaks obtained in previous work by Raj and Jayalakshmy (2015) when they succeed in synthesizing ZnO from the root extract of *Zingiber Officinale* (Raj and Jayalakshmy 2015).

#### UV-Vis absorption analysis

UV-Vis absorption spectrum analysis in this study was used to confirm the ZnO-NPs formation (B. Kumar et al., 2014). Concurrently, it was possible to monitor the stability of the biosynthesized nanoparticles (Zhang et al., 2016). Fig. 5 presents the absorption peak of the ZnO-NPs around 370nm which was in the

range of characteristic peak of ZnO-NPs from previous study (Khorsand Zak et al. 2011). It was specified that the ZnO-NPs exhibited exciton absorption at 370nm, owing to their large exciton binding energy at room temperature. Absorption in the wavelength of 370 nm was further confirmed that the absorption spectrum was slightly blue shifted, pertaining to the bulk value (377 nm) of ZnO-NPs. The shifted line in the absorption edge was due to the quantum confinement effect between the individual nanoparticles (Elumalai et al., 2015). According to Gupta et al. (2015), the reducing size of the nanoparticle generally could be recognized with the absorption edge that systematically shifted to the lower wavelength or higher energy (Gupta et al. 2015).

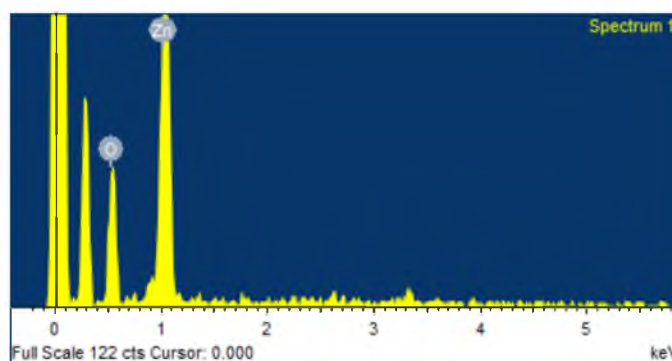


Fig.4 EDX spectrum of biosynthesized ZnO-NPs.

Table 1 Elemental analysis by EDX for ZnO-NPs.

Component	Weight%	Atomic%
O	31.62	65.39
Zn	68.38	34.61

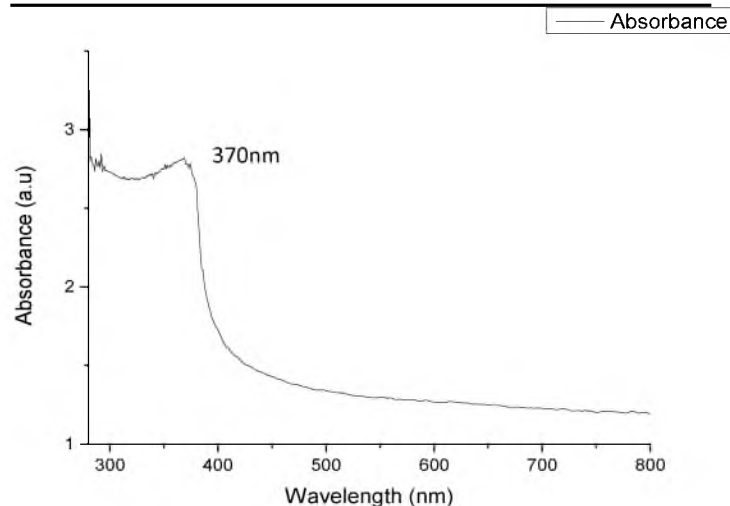


Fig. 5. UV-Vis absorbance of ZnO-NPs.

#### Evaluation of antibacterial activity

The antibacterial properties of ZnO-NPs have been analyzed by agar diffusion methods and the results were shown in Fig. 6. The antibacterial activity of ZnO-NPs against bacterial strains of *E. coli* ATCC 25922 was measured as area of inhibition zone. The inhibition zone could be clearly observed in the biosynthesized ZnO-NPs and commercial ZnO-NPs which proved the antibacterial properties of both nanoparticles. In addition, the zone of inhibition produced by the biosynthesized ZnO-NPs was larger than the commercial ZnO-NPs which was in correlation to the previous work done by Gunalan, Sivaraj, and Rajendran (2012) which reported that the inhibitory effect of the green synthesized nanoparticles was bigger than chemically synthesized nanoparticles. Table 2 presents the inhibition area of the

samples ( $\text{mm}^2$ ). The inhibition areas of the commercial ZnO, biosynthesized ZnO<sup>a</sup> and biosynthesized ZnO<sup>b</sup> were 18.76  $\text{mm}^2$ , 24.704  $\text{mm}^2$  and 51.428  $\text{mm}^2$ , respectively. Other than that, the percentage differences of the biosynthesized ZnO<sup>a</sup> and biosynthesized ZnO<sup>b</sup> were 31.5% and 173.84%, respectively in comparison to commercial ZnO. It was proved that the biosynthesized ZnO was superior in terms of antibacterial properties as opposed to ZnO-NPs. Also, it was shown in the earlier study by Wahab et al., (2010) that by increasing the concentration of ZnO-NPs in wells and discs, the growth inhibition has also been increased (Wahab et al., 2010). In addition to that, the size of inhibition zone was affected by various factors such as the type of bacteria, the size, and the concentrations of ZnO-NPs (Zarrindokht Emami-Karvani, 2012)



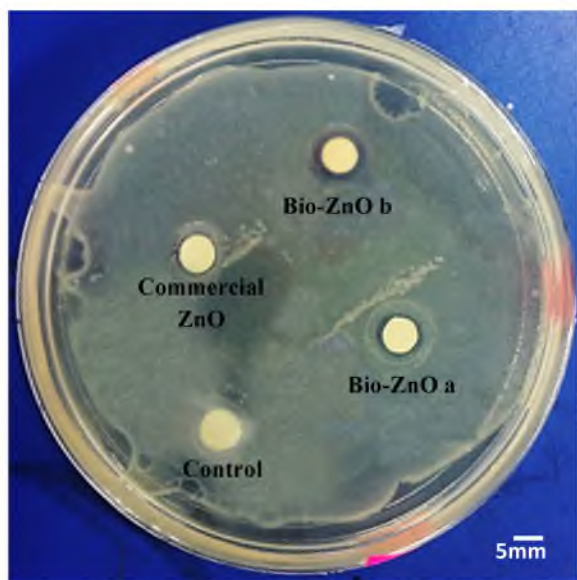


Fig. 6. Antibacterial activity of ZnO-NPs.

Table 2. Inhibition area of ZnO-NPs.

Component	Inhibition area (mm <sup>2</sup> )	% difference with commercial
Control	0	
Commercial ZnO	18.78	
Biosynthesized ZnO <sup>a</sup>	24.704	31.5
Biosynthesis ZnO <sup>b</sup>	51.428	173.84

## CONCLUSION

The rapid biological synthesis of ZnO-NPs using a fruits extract of *Ananas Comosus* as reducing agent was successfully conducted and the results proved that the biosynthesized ZnO was simple, eco-friendly, cost effective and offered pertinent route for synthesis of nanoparticles. The size of synthesized nanoparticles ZnO was in the range of 30-57nm. The morphology of the ZnO-NPs was characterized by using FESEM. EDX analysis revealed that the purity of ZnO was in line with the XRD result. The absorbance obtained from the UV-Vis spectrum analysis also showed the absorption peak of the ZnO-NPs is at around 370nm. It was proven in this study that the biosynthesized ZnO-NPs were acted as an effective antibacterial agent against common pathogenic microorganisms. It was also believed that the use of plant extracts in the synthetic procedure resulted in enhanced antibacterial and antioxidant properties of ZnO-NPs. Therefore, it could be concluded that the green synthesis of ZnO-NPs using *Ananas Comosus* fruits extract has a tremendous potential as an alternative to chemical methods.

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