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Original Research Article

Microbiologically Induced Corrosion (MIC) of Carbon Steel in Biodiesel: a Comparative Analysis

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Abstract

The damaging process known as microbiologically influenced corrosion (MIC) can be triggered by the bioactivities of microorganisms. The occurrence of this phenomenon can be attributed to the presence of biodiesel in carbon steel diesel mixture storage tanks, since the biodegradability of the fuel promotes microbial development and the MIC process. In this work, the effects of different biodiesel concentrations (B0, B15, B20, B30, and B100) on biocorrosion in ST-37 carbon steel caused by three species were investigated. Some of the species confirmed to be involved are *S. marcescens*, *B. megaterium* and *B. lichenisformis*. These three species are undoubtedly able to survive in a biodiesel-based media. In addition to producing EPS, a species that may slow the rate at which carbon steel corrodes, biodiesel can be utilised as a supply of nutrients. Nonetheless, the diverse life cycles of microbes have the potential to accelerate corrosion of carbon steel. The average corrosion rate with the effect of *B. lichenisformis* is lower than the other two species, notably in the B100, with fewer colonies than *S. marcescens* and *B. megaterium*.Some of the species confirmed to be involved are *S. marcescens*, *B. megaterium* and *B. lichenisformis*.

Keywords: Corrosion; biofilm; hydrocarbons; biofuels; microorganism

1. Introduction

Petroleum-derived hydrocarbons are significant source of energy, but the demand for fossil fuels is rising in parallel with them. This contrasts with its availability, which is becoming getting harder to obtain. The rate of growing vehicle ownership is one of the factors behind the global scarcity of fossil fuels, particularly in Indonesia. As a result, in order to address the petroleum issue and prevent the depletion of petroleum fuels, biofuels are needed as alternative energy sources. Biodiesel is one kind of biofuel that is currently in high demand. It is possible to make biodiesel from soybeans, jatropha, and palm oil. In Indonesia, plants such as palm oil, soybeans and jatropha are widely grown and have a lot of potential for development in response to the country's growing needs. Diesel oil and biodiesel are still combined at the moment. Research on the blend of diesel and biodiesel fuel in Indonesia has remained ongoing due to its expanding need for diesel fuel in the transportation and industrial sectors. By 2025, the Indonesian government hopes to have up to 30% of its fuel consumption come from biodiesel blends, as stated in Permen ESDM No. 12 2015.

The typical storage tank for petroleum products and biofuels is a sizable carbon steel tank. The industry uses carbon steel more frequently for storage tanks and as a building material. Since carbon steel is less expensive than other metals like stainless steel, it is used in construction (Heyer et al., 2013).

Typically, big tanks are used to store petroleum products and biofuels, which are subsequently pumped through pipelines to smaller tanks for storage or transportation. There is a chance that microorganisms will contaminate during distribution. Although biodiesel in its pure form is chemically stable, it degrades during shipping, storage, and use due to moisture absorption, microbial oxidation, and condensed water contamination. This is the main cause of corrosive activities (Demirbas, 2009; Eleryan et al., 2024; Eryilmaz and Yesilyurt, 2016). It is extremely difficult to prevent product contamination by microorganisms since sterile conditions cannot be maintained during fuel transportation and storage because microorganism might degrade fuel (Lee et al., 2010). The development of these microorganisms contributes to corrosion on the tank (microbiologically influenced corrosion (MIC)).

Although microbes can consume hydrocarbons as a source of nutrition, corrosion in the tank is possible. Additionally, biofuels are an appropriate medium for microorganism growth. Extracellular polymeric substances (EPS), organic, and inorganic acids—products of MIC metabolism—form biofilms that have the ability to alter environmental conditions and initiate corrosion. The electron transfer process on the metal surface is impacted by microorganisms, which can cause the corrosion reaction to proceed more quickly or more slowly (Heyer et al., 2013). Corrosion caused by MIC can found on the inner surface, bottom of the storage tank contains fuel oil. MIC typically coexists with other forms of corrosion. Sludge (slime, biofouling, or biofilm) on the inside and bottom of the tank indicates the presence of fuel, and the MIC is typically found in tanks storing crude oil (Groysman, 2014). Numerous investigations have shown that carbon steel MIC has a major detrimental effect. As sulphides from SRB metabolism settle on the surface of iron and form iron sulphide (FeS) at oil field separators and offshore installations, MIC and biofilm may be responsible for pitting (Dunne jr, 2002; Wikieł et al., 2014). Moreover, because of the anaerobic conditions, SRB can form biofilms in the bottom of fuel and crude oil tanks, which show up as a black layer (Welikala et al., 2024).

One of the most frequent microbes linked to the deterioration of the petroleum production system is the anaerobic SRB (Von Wolzogen Kuehr and van der Vlugt, 2006). Recent research, however, indicates that biocorrosion may also be caused by different kinds of microorganisms (Jan-Roblero et al., 2008; Muthukumar et al., 2003; Rajasekar et al., 2005; Zhu et al., 2003). Numerous species, including *S. marcescens*, are capable of breaking down a wide range of hydrocarbon. These organisms predominate in the corrosion result of the napha piping transport here, following *Bacillus sp.* (Rajasekar et al., 2007). In this study, the effects of the three species types on the corrosion of carbon steel at different concentrations of biodiesel will be compared. Given the increasing use of biodiesel in combination with diesel oil, it was necessary to investigate the characteristics of carbon steel on MIC, including the proportion of bacteria adhering to the biofilm and the rate of corrosion. Therefore, this study evaluates the effects of different microbe species that are often found in biodiesel media on the rate at which carbon steel corrodes. At some point it is clear which species is the more prevalent and significant.

2. Methods

In order to observe the MIC characteristics of carbon steel ST-37 in a mixture of 15% (B15), 20% (B20), 30% (B30), and 100% (B100) v/v biodiesel containing *S. marcescens*, *B. megaterium*, and *B. lichenisformis*, preparation, experimentation, and corrosion analysis were the three primary phases of this study.

2.1 Preparation

The culture step, test solution preparation, metal preparation, and reactor preparation were components of the preparation stage. Bushnell Haas (BH) was the growing medium used for microorganism. $MgSO_4$ 0.2 g/L, $CaCl_2$ 0.02 g/L, KH_2PO_4 1 g/L, K_2HPO_4 1 g/L, NH_4NO_3 1 g/L, and $FeCl_3$ 0.05 g/L made up the BH medium. Until the microorganism reached a stationary growth stage, these bacteria were maintained in a sterile BH medium with a 100 mL working capacity in order to increase the number of bacterium. Following the entry into the stationary phase, the bacteria were acclimated by adding 2 mL of inoculated medium to 300 mL of BH medium and 1 g of sterilised diesel oil as the only

nutrition in an Erlenmeyer. Later that, the mixture had been incubated for 30 days at 200 rpm and 27 0C in a rotary shaker.

The primary experimental mediums for the study were chosen to be biodiesel and petroleum diesel. Petrodiesel (B0) is Pertamina DEX, a commercial petrodiesel from PT Pertamina, an Indonesian company, with cetane number 53. In the meantime, PT Darmex Agro supplied commercial palm oil biodiesel (B100). To remove potential contaminants and microorganisms, the hydrocarbon intended for use as the immersion media was filtered using a CA filter membrane with a pore size of 0.45 µm.

Preparing the specimen involved multiple steps. As per the ASTM G 31-72 standard, the carbon steel specimen was cut into 1 cm by 1 cm pieces. The specimens were polished using 240–1200 grid abrasive paper in accordance with ASTM G 1-81, and contaminants and fat were removed by washing them in water and ethanol. The samples were preserved dry in a desiccator after being washed and dried. Acrylic was used for the reactor lid, while glass was utilised for the specimen hanging pole and the soaking reactor.

2.2 Experimental

Diesel oil was combined with the inoculum medium, which contains each species microorganism, to create an immersion medium with a volume ratio of 10% to 90%. The immersion medium has a working capacity of 200 mL. The carbon steel coupons were submerged in the immersion medium's oil phase and suspended on a hook. The "solution volume to specimen area" ratio, as specified by ASTM G 31–72, must be between 0.20 and 0.40 mL/mm^2, meaning that the maximum coupons with four coupons with dimensions of 1 cm by 1 cm by 0.1 cm and a working capacity of 200 mL were used. After being waxed and covered to prevent contamination, the reactor was set up at ambient temperature and air pressure. In this study, extended soaking periods of 10 and 20 days were employed.

2.3 Corrosion analysis

This study included some analysis, such as the total plate count (TPC) method for analysing the bacterial colonies on biofilm and gravimetric method using ASTM G1 for monitoring the rate of corrosion and the impact of microorganism on corrosion. Two specimen replicas for each analysis method were evaluated.

3. Result and Discussion

3.1 Growth of microorganism

Bacteria use the process of biofilm development to adapt to their surroundings. Since biofilms are one of the most popular survival strategies used by microorganisms, microorganisms that were initially suspended in the medium would adhere to metal surfaces and form biofilms. This allows microorganisms species to modify their surroundings as a defence mechanism (Heyer et al., 2013). Microorganisms prefer to adhere to metal surfaces because there are more nutrients accessible there than on metal surfaces (Characklis and Marshall, 1990). As a result, the microorganisms trap the necessary nutrients inside the biofilm. Analysis using the TPC method was carried out to count the number of colonies in the biofilm that was formed. The number of colonies in the biofilm was counted during the soaking period to be able to obtain the growth profile of *S. marcescens*, *B. megaterium* and *B. lichenisformis* on biofilms attached to carbon steel metal surfaces. Furthermore, the ability of each species to survive in the biofilm that forms on metal surfaces was ascertained by performing a quantitative study on colonies in biofilms. The number of *S. marcescens*, *B. megaterium* and *B. lichenisformis* colonies that have been counted can be seen in Figure 1,2 and 3.

In Figure 1, it can be seen that in general the growth of *S. marcescens* was quite stable during the 20 day soaking period at various biodiesel concentrations. There were no significant increases or decreases. In the first 10 days, the average number of colonies in the B15 variation reached 207.5 x 10^5 CFU/cm^2. A slight increase occurred 10 days later, reaching 290 x 10^5 CFU/cm^2. A slight decrease occurred in the concentrations of B20, B30 and B100. In the first ten days the average number of colonies for B20,

B30 and B100 concentrations was 220 x 10^5 CFU/cm^2, 165 x 10^5 CFU/ cm^2, and 340 x 10^5 CFU/ cm^2,. On day 20 the average number of colonies reached 127.5 x 10^5 CFU/ cm^2, 107.5 x 10^5 CFU/ cm^2, and 192.5 x 10^5 CFU/ cm^2. On the other hand, Figure 2 shows the growth phenomenon of *B megaterium* which is not much different from *S. marcescens*. The number of B megaterium colonies will increase from 142.5 x 10^5 CFU/ cm^2 to 232.5 x 10^5 CFU/ cm^2 during the 20 day soaking period for varying B15 concentrations. For concentrations of B20, B30 and B100, the number of *B megaterium* colonies will decrease to 102.5 x 10^5 CFU/ cm^2, 270 x 10^5 CFU/ cm^2 and 342.5 x 10^5 CFU/ cm^2 on day 20 from the average number of colonies of 265 x 10^5 CFU/ cm^2, 447.5 x 10^5 CFU/ cm^2, 502.5 x 10^5 CFU/ cm^2 on day 10.

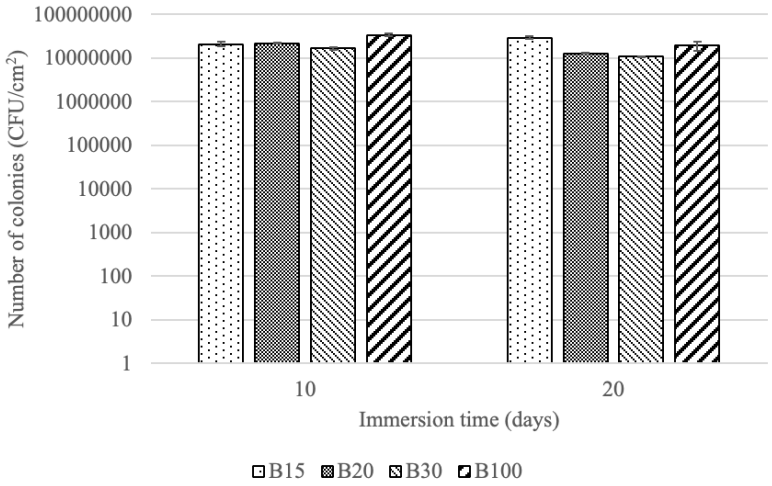

Figure 1. Quantification of *S. marcescens* growth on the biofilm at different times and concentrations of biodiesel

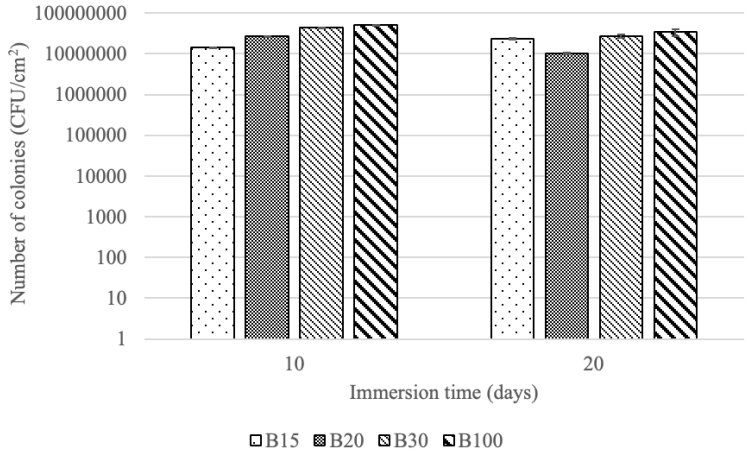

Figure 2. Quantification of *B. megaterium* growth on the biofilm at different times and concentrations of biodiesel

Compared to *S. marcescens* and *B. megaterium*, the growth of *B. licheniformis* generally increased significantly for each variation in biodiesel concentration (Figure 3). However, in general, the average number of *B. licheniformis* colonies is less than that of the other two species. On the tenth day, the average number of viable *B. lichenisformis* colonies was 4330 CFU/cm^2 and 916 CFU/cm^2, respectively, at various concentrations of B15 and B20. There were 2083 CFU/cm^2 surviving colonies, which is fewer than the B30 variety for the same soaking day. The B100 has the greatest number of viable colonies 91 x 10^3 CFU/ cm^2. Ten days later, each variation will experience an increase in the number of living colonies, reaching 13.6 x 10^4 CFU/ cm^2, 22.9 x 10^4 CFU/ cm^2, 22 x 10^5 CFU/ cm^2 and 26.6 x 10^4 CFU/ cm^2. At the end of the 20 day soaking period, the highest number of *B. licheniformis* colonies are alive in the B30 variation.

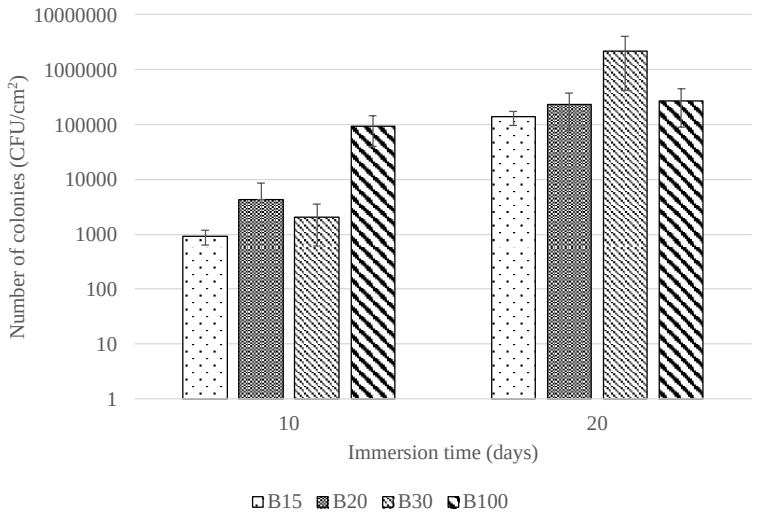

Figure 4. Quantification of *B. lichenisformis* growth on the biofilm at different times and concentrations of biodiesel

The life cycle of the microorganisms in the biofilm provides an insight into the phenomenon of fluctuations in the quantity of living microorganisms. Single-cell bacteria on metal surfaces covered in a thin film layer communicate and attach to one another to create microbial colonies. Because the passive coating of iron oxide on carbon steel provides the ideal surface for microbe attachment, carbon steel is a type of metal that corrodes easily (Heyer et al., 2013). This study refer to the process as attachment or adhesion. While EPS influences the attachment process of the cell and results in a tight connection between bacteria and the surface, electrostatic interactions also play a role in the attachment of microorganisms to surfaces (Wikieł et al., 2014) The joining of other bacteria (colonisation) will strengthen the interactions between microorganism and cause them to accumulate, as evidenced by the complexity of the biofilms that are generated rising (Dunne jr, 2002; Pusparizkita et al., 2023). The early stage of biofilm maturation is the following process. All of the metabolic activities of colonised cells are being unified, which includes each bacterium combining its structure, activity, and function into the biofilm (Rao, 2012) and using substrates and nutrients in the medium. The colony seems larger at this stage because to a process of polysaccharide breakage, expansion, and maturation, which also increases the thickness of the biofilm (Heyer et al., 2013). An increase in the number of colonies can be used to characterise this phase when analysing the outcomes of colony counts. In the last stage, some bacteria may spread and settle in new areas, which could result in a drop in the number of colonies (Monroe, 2007). The accumulation of waste materials and nutrient depletion surrounding the biofilm play a major role in the release process, which happens when bacteria exit the biofilm (Toyofuku et al., 2016). Microorganisms can start the production of biofilms on the surface of other metals through the release process.

3.2 Corrosion rate

Figure 4 to 6 depicts the average rate of corrosion caused by *S. marcescens*, *B. megaterium*, and *B. lichenisformis* activity at different concentrations of biodiesel. As a result, the average rate of carbon steel corrosion under the impact of microorganism activity will decrease. The average corrosion rate of carbon steel for variations of the *S. marcescens* species at the B100, however, is larger than changes at other concentrations. This is where things diverge. In the meantime, the B15 and B20 variants had the highest rate of carbon steel corrosion in the *B megaterium* during the first 10 days of immersion; however, during the next 20 days, the B20 and B100 variations had the highest rate of corrosion. There is little deviation in the value. In contrast to the other two species, *B lichenisformis*' activity offers a unique

representation of the corrosion rate. Compared to other biodiesel concentration changes, the greatest concentration, B100, actually exhibits a low corrosion rate. This can be explained by the fact that none microorganisms can have the same life cycle. On the other hand, the day 20 reduction in the rate of corrosion indicates that the biofilm that had developed had protective qualities. The thicker protective biofilm formed from B100 products may be the reason why the specimen submerged in pure biodiesel for the *B. lichenisformis* variant corrodes at a slower rate than mixed variations (B15, B20, and B30).

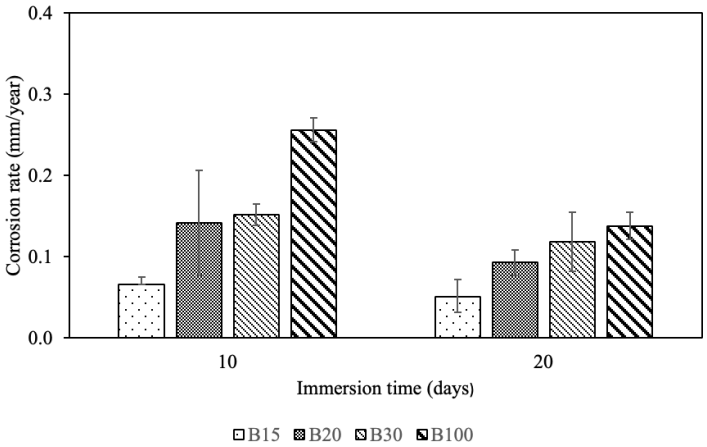

Figure 4. *S. marcescens* corrosion rate on carbon steel ST 37 at different times and concentrations of biodiesel

Still, there are instances in which differences in the effect of *S. marcescens* lead to high corrosion rates in the B100 environment. Considering biodiesel is more hygroscopic than conventional diesel, diesel mixtures containing biodiesel might include a higher percentage of water (Pusparizkita et al., 2021). Despite being mostly made up of saturated and unsaturated fatty acids, biodiesel eventually becomes corrosive because of its water content, which encourages microbial development. This growth may hydrolyze the esters in biodiesel, producing additional corrosive fatty acids at the biodiesel or water interface (Adama et al., 2024). In conditions where the biofilm has not yet formed completely, the hygroscopic condition of biodiesel can accelerate the corrosion reaction on the carbon steel surface.

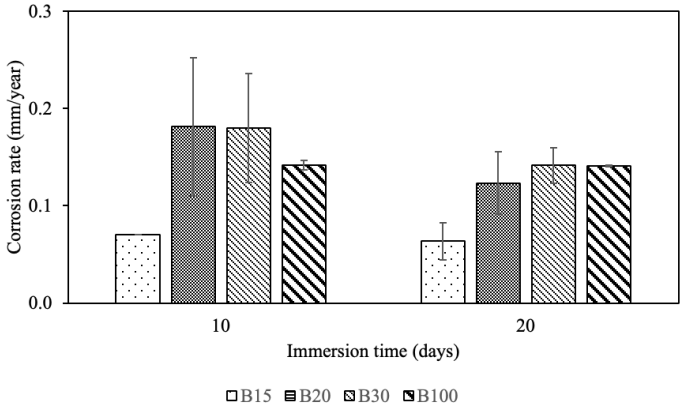

Figure 5. *B. megaterium* corrosion rate on carbon steel ST 37 at different times and concentrations of biodiesel

This is supported by the results of XRD analysis which found corrosion products in the form of magnetite in systems involving biodiesel (Fardilah et al., 2022; Pusparizkita et al., 2021, 2020, 2018). In total, four water molecules are reduced to hydrogen and three Fe(0) atoms are oxidised to produce one Fe(II) cation and two Fe(III) cations. It can be written as:

$$3Fe + 4\,H_2O \rightarrow Fe_3O_4 + 4H_2$$

Sometimes extremely thick biofilm circumstances coexist with the activities of microorganisms that may produce acid metabolites and medium conditions that may accelerate the rate at which carbon

steel corrodes when they combine. The variance of *B. megaterium* on B20 and B30 medium on day 10 illustrates this phenomenon (Figure 5). The formation of metabolites such as organic acids can compound the MIC that carbon steel is susceptible to by oxidising the steel. *Bacillus*, which are sometimes thought of as acid-producing bacteria and sometimes as slime-forming bacteria, are able to make organic acids and extracellular polymeric substances (EPS) (Wu et al., 2015), is commonly found in the diesel mixture that may assist in hydrocarbon biodegradation and biocorrosion in the metal of the diesel storage tank (Aslan et al., 2022; Pusparizkita et al., 2021). Moreover, microorganisms have the ability to release long-chain organic acids from the hydrolysis of biodiesel and short-chain organic acids from the tricarboxylic acid cycle, which enhances the acidity of the diesel solution and speeds up corrosion. (Roberge, 2008).

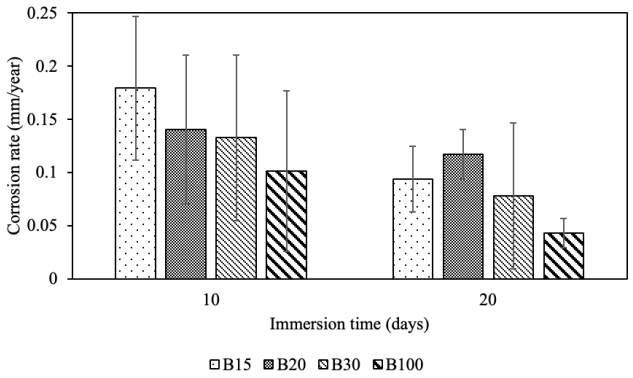

Figure 6. *B. lichenisformis* corrosion rate on carbon steel ST 37 at different times and concentrations of biodiesel

4. Conclusions

The cycle of microorganism development has a significant impact on the rate of corrosion, either increasing or decreasing. Every kind of microorganism has a unique life phase length. The three species found biodiesel to be particularly beneficial for growth, with the B100 variety having the largest average number of microbe colonies. The activity of microorganisms will also slow down the rate of corrosion, in addition to the duration of soaking. The development of biofilm on the carbon steel surface provides a means of understanding this process. However, additional research necessitates taking SEM images of the morphology and appearance of the biofilm generated for each species and comparing them.

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