Disinfection Performance against *Salmonella* Typhi in Water by Radio Frequency Inductive Couple Plasma System

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Abstract. The disinfection performance of the radio frequency inductively coupled plasma (RFICP) system against *Salmonella* Typhi in water was examined using continuous flow experiments. The evaluation was based on disinfection efficiency, death rate constant, disinfection yield, and energy consumption. For all experiments the electromagnetic flux generated in the plasma reactor varied from 4 to 6 W/cm^2. The disinfection efficiency and death rate constant of *Salmonella* Typhi decreased with the increase of the initial number of *Salmonella* Typhi bacteria. The disinfection yield increased from 784 to 1889 CFU/KWh and the energy consumption decreased from 0.28 to 0.07 KWh/L with the flowrate increasing from 5 to 20 mL/minute. The flowrate is an important parameter in predicting disinfection performance against pathogenic bacteria in water to design drinking water treatment plants.

Keywords: *disinfection; pathogenic bacteria; radio frequency inductively coupled plasma system;* Salmonella *Typhi; water treatment.*

1 Introduction

Salmonella Typhi causes intestinal illnesses such as typhoid and paratyphoid fever. *Salmonella* Typhi bacteria are classified as a food-borne pathogenic bacteria; they spread through drinking water or tap water. *Salmonella* Typhi bacteria cause human deaths all over the world, resulting in a notification rate of 20.4 cases per population of 100,000 [1]. *Salmonella* Typhi is commonly detected in animal-food products such as eggs, poultry and meat [2], fresh vegetables and processed fruit [3]. *Salmonella* is also detected in surface water and drinking water [1]. The number of pathogenic bacteria has to be minimized in the source of contamination. To reduce the number of pathogenic bacteria, chlorine compounds are commonly used as sanitizing agents in drinking water treatment and in production processes, however, some researchers do not recommend the use of chlorine for this purpose [4]. Chlorine in water can react

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with other natural compounds to form trihalomethanes (THMs) as chlorination byproducts. Cancer researchers have found that THMs trigger the production of free radicals in the body, causing cell injury, and that they are highly carcinogenic, even at low concentrations. They are the cause of the majority of human cancers in the world.

Therefore, research to reduce the use of chlorine in drinking water treatment is of great importance. Alternative technologies in drinking water treatment processes have been investigated using physical methods such as a membrane bioreactor [5], pulsed electric field irradiation [6], and floating media filter-microfiltration [7]. The advanced oxidation process (AOP) is an alternative process for removing pathogenic bacteria from water. This process generates radical species such as $^{\bullet}OH$, $H^{\bullet}$, O_3, H_2O_2 that have a high oxidation potential and can also produce ultraviolet light and shockwaves, which can kill pathogenic bacteria in water. Many kinds of AOP processes have been investigated to remove total coliforms and fecal coliforms from water, such as pulsed high voltage [8], photodynamic disinfection [9], and graphite adsorbent electrochemical disinfection [10]. In our previous studies we found that the removal efficiency of total coliforms and fecal coliforms using pulsed high voltage (PHV) and dielectric barrier discharge (DBD) is higher than that of the radio frequency plasma system [11,12]. The problem with the application of PHV and DBD is that the surface of the electrodes suffers from scale build-up because of minerals in the water.

Compared with other advanced oxidation processes, the radio frequency inductive couple plasma (RFICP) system is a new technology for the disinfection of microorganisms in water without chemical disinfection [6-9,11,12]. To the best of our knowledge, after an intensive literature review, the present study is the first one to focus on the removal of *Salmonella* Typhi using RFICP. The results obtained may be of great reference value to advanced drinking water treatment. Accordingly, the aim of this research was to study the capability of the radio frequency inductive couple plasma (RFICP) system to remove *Salmonella* Typhi from water by carrying out continuous flow experiments. The effects of the initial number of *Salmonella* bacteria and the flowrate on the removal of *Salmonella* Typhi were studied. Based on the experimental data, the disinfection efficiency, the death rate constant, the disinfection energy consumption and electromagnetic flux were calculated.

2 Material and Methods

2.1 Source of Water and *Salmonella* Typhi

Water was collected from a spring at Mount Talang, West Sumatra Indonesia. The physicochemical properties and microbiological parameters of the spring water are displayed in Table 1. The typhi O antigen of *Salmonella* (Accucare, India) was used as the source of *Salmonella* Typhi and was inoculated in *Salmonella* brilliant agar and incubated at 37 °C for 24-48 hours.

Table 1 Physicochemical properties and microbiological parameters of spring water.

Parameters	Unit	Values
Physicochemical properties		
Electric Conductivity (EC)	μS/cm	156
Total Dissolved Solid (TDS)	mg/L	12
pH	-	6.5
Turbidity	NTU	0.16
Iron (Fe)	mg/L	0.12
Manganese (Mn)	mg/L	0.13
Sulfate (SO_4)	mg/L	0.23
Total Phosphor	mg/L	ND
Magnesium (Mg)	mg/L	1.50
Microorganisms		
Total *coliforms*	CFU/100 mL	100
Fecal coliforms	CFU/100 mL	20
Salmonella Typhi	CFU/100 mL	0

2.2 Experimental Set-up

The experimental set-up of the RFICP system utilized in the present study is displayed in Figure 1. The glass reactor had a thickness of 2 mm, a length of 30 cm and a diameter of 1 cm. It was wrapped in copper wire ($\varnothing$ 2 mm). The RFICP discharge plasma system consisted of a radio frequency (RF) generator and an inductive couple plasma (ICP) reactor as load or plasma reactor. In the system, a high-frequency electric current produces by the RF generator passes through a solenoid coil producing an axial magnetic field in the ICP, which induces the vortex electric field maintaining the RFICP discharge plasma.

The electric field is proportional to the RF, based on the Maxwell equations, while the magnetic field in the ICP discharge plasma is also determined by the current in the coil. The ICP load is influenced by electrical parameters such as resistance and inductance. The system was equipped with a high-voltage probe (Tektronix P6015A, Tektronix Inc., USA), a current probe (Tektronix P6022, Tektronix Inc., USA) and a Picoscope 4424 with four channels (Picotech, United Kingdom). The typical voltage and current generated by the RF generator is displayed in Figure 2.

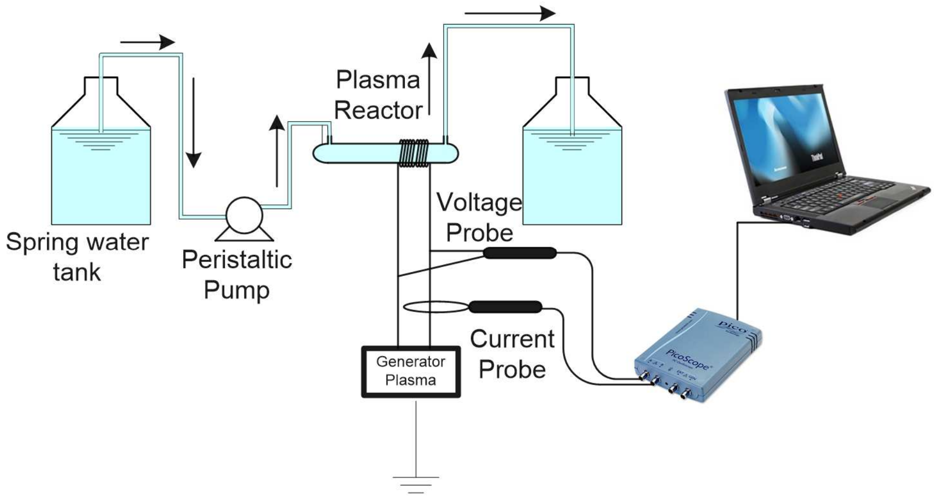

Figure 1 Experimental set-up of RFICP system.

2.3 Experiments

Two series of continuous experiments were conducted to investigate the disinfection performance of the RFICP system against *Salmonella* Typhi. The first series examined the effect of the initial number of *Salmonella* Typhi. In order to vary the initial number of *Salmonella* Typhi, 1, 2 and 3 drops (each drop is around 10 μL) of the inoculated *Salmonella* Typhi were added to 600 mL of water. The number of *Salmonella* Typhi bacteria in the resulted samples equaled 28, 40 and 44 CFU/mL respectively. In our previous studies using RFICP in batch experiments, 100% disinfection efficiency of total coliforms and fecal coliforms was obtained with applied frequency at 3.7 MHz, after running more than 5 minutes [11,12].

Based on the results of the batch experiments, the hydraulic retention time was set at 20, 10 and 5 minutes. These values correlate to the flowrate at 5, 10 and 20 mL/minute in the second series to investigate the effect of the flowrate and was controlled with a peristaltic pump. The applied frequency was set at 3.7 MHz. Effluent from the plasma reactor was sampled every 10, 30, 60, 90 and 120 minutes and analyzed for the number of *Salmonella* Typhi bacteria. The temperature was stable during the experiments, with values of 28.8±0.12 °C. The pH was also stable in the range of 6.5-6.7.

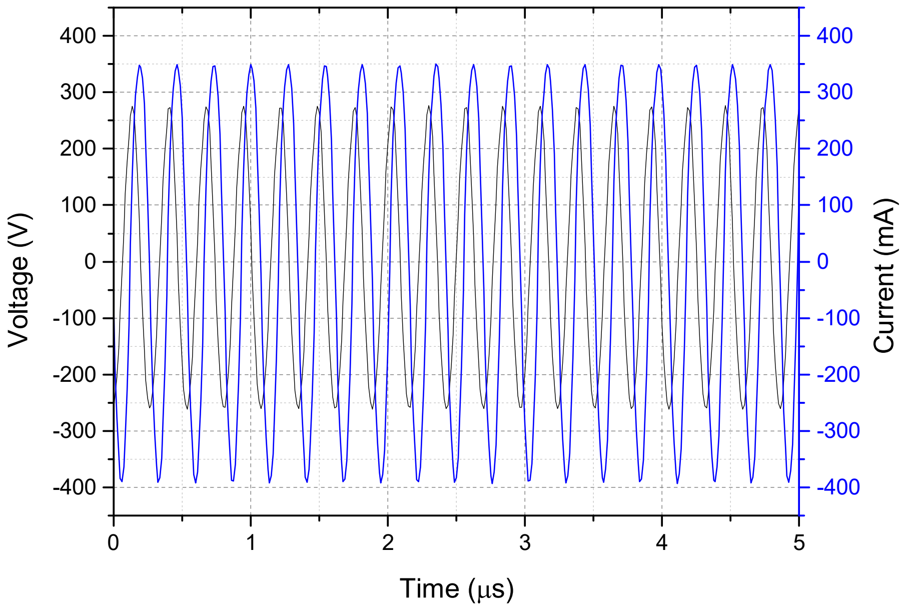

Figure 2 Waveforms of voltage and current generated by RFICP. The frequency was set at 3.7 MHz.

2.4 Analysis

The applied voltages and discharge currents were monitored using the oscilloscope and through the high-voltage probe and the current probe. *Salmonella* Typhi was measured according to the International Organization for Standardization 19250:2013 standard method [13]. To make 10^1 dilutions, 0.5 mL of water sample was added into a tube containing 4.5 mL of normal saline water. Then, the tube was carefully mixed on a vortex for one minute.

Agar plates were prepared by adding 28.1 g of nutrient agar (brilliant *Salmonella* agar by Oxoid, United Kingdom) to 1 L of ultra-pure water. A 1-mL dilution water sample was mixed in 5 mL of melted agar broth and poured onto a petri dish (diameter 5 cm). The petri dish was kept overnight at 37 °C for 24 h in an incubator. The purple colonies that formed were counted the total number of *Salmonella* Typhi as colony forming unit (CFU/mL).

3 Results and Discussion

3.1 The Electromagnetic Flux

The electromagnetic flux (S) in the ICP was calculated using formula in Eq. (1) as follows [18]:

$$S = 9.94 \cdot 10^{-2} \cdot (I.n)^2 \sqrt{\frac{f}{\sigma}} \tag{1}$$

where S is electromagnetic flux (W/cm^2), I is current (A), n is number of turns per unit length (turns/cm), f is frequency (MHz), and σ *is* electric conductivity (ohm^{-1} cm^{-1}). In this study, the electromagnetic flux generated in the plasma reactor varied from 4 to 6 W/cm^2 during the experiments. These values are similar with our previous results [11,12] for disinfection of fecal coliforms and total coliforms using RFICP system.

3.2 Profile of Disinfection Efficiency of *Salmonella* Typhi

The profile of the disinfection efficiency of *Salmonella* Typhi is displayed in Figure 3a. For all runs, the disinfection efficiency of *Salmonella* Typhi decreased with 28%, 25% and 13.7% with the initial number of *Salmonella* Typhi bacteria at 28, 40 and 44 CFU/mL, respectively, after running 10 minutes. After 60 minutes, a stable decreasing trend was observed until 120 minutes. Over the whole running period, the maximum disinfection efficiency with the initial number of *Salmonella* Typhi at 28, 40 and 44 CFU/mL was 75, 55 and 48%, respectively. The result indicates that the disinfection of *Salmonella* Typhi depends on the initial number of *Salmonella* Typhi bacteria.

In drinking water treatment plants, the flowrate plays an important role in controlling the disinfection performance against pathogenic bacteria. For this reason, continuous flow experiments were conducted in the present study, to see the effect of flowrate on disinfection efficiency of *Salmonella* Typhi, as shown in Figure 3b. The number of *Salmonella* Typhi bacteria in the effluent decreased with time over the first period. After 120 minutes, the disinfection efficiency of *Salmonella* Typhi bacteria was 55, 45 and 36% with flowrate at 5, 10, 20 mL/minute, respectively. The results indicated that the number of *Salmonella* Typhi bacteria in the effluent increased with an increase of the flowrate.

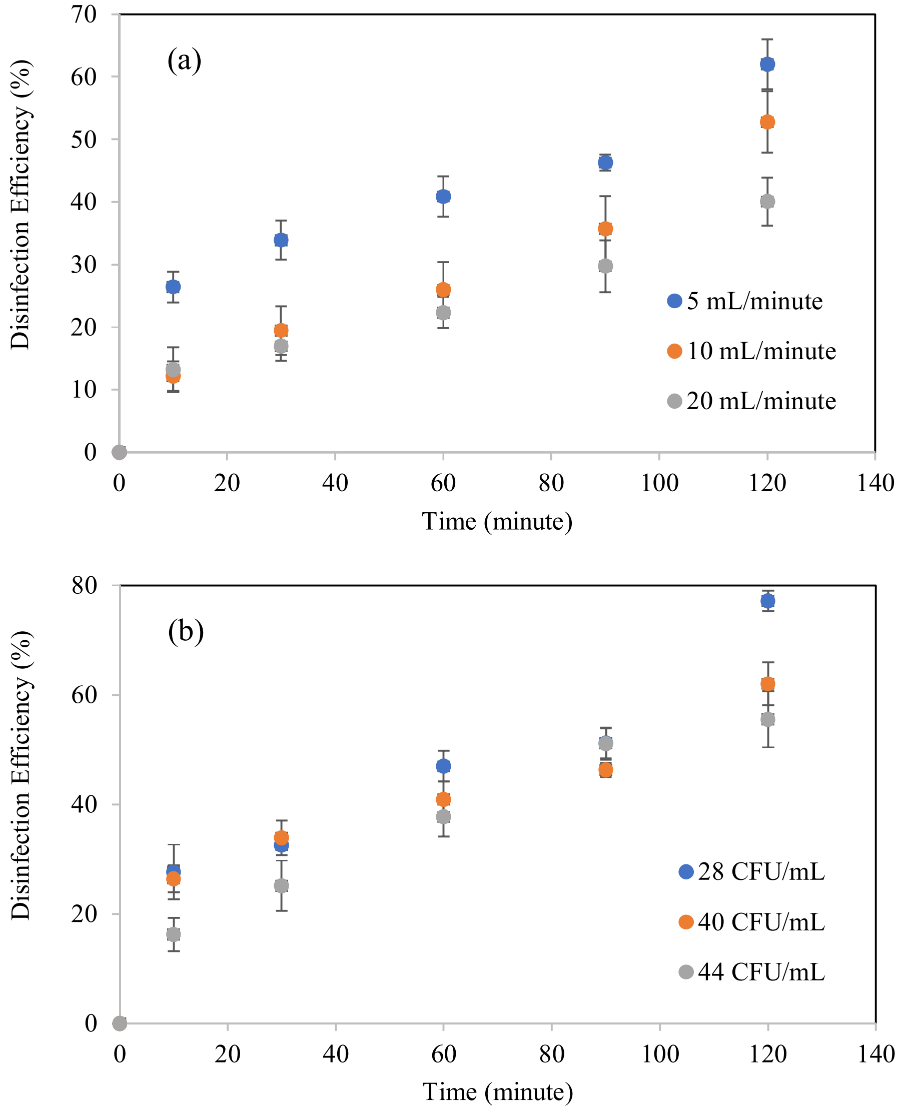

Figure 3 Profile of the disinfection efficiency of *Salmonella* Typhi at applied frequency 3.7 MHz: (a) variation of flowrate with the number of *Salmonella* Typhi at 40 CFU/mL, (b) variation of the number of *Salmonella* Typhi with flowrate 5 mL/minute. All the data represented in triplicate.

Plasma can produce active species, such as O_3, H_2O_2 and •OH by electron impact dissociation of oxygen and water molecules, followed by chemical reactions during the plasma discharge process in humid air [14]. These active species can dissolve into the solution and further degrade pollutants via oxidation processes [15,16]. In addition, UV light and shock waves produced by the plasma discharges may also play a role in the degradation of pollutants and the disinfection of microorganisms in aqueous solutions [15,17]. The disinfection efficiency (DE) of microorganisms depends on the interaction between pathogenic bacteria and colloidal particles, as reported by Chiemchaisri, *et al.* [7]. They also found that a combined process of coagulation, floating media and microfiltration is highly effective in removing particles, total coliforms and fecal coliforms, while it does not contribute significantly to the removal of coliphage virus.

The disinfection efficiencies using this system obtained were in the ranges of 96-98%, 52-98%, 68-75% for fecal coliforms, total coliforms and coliphage virus, respectively. However, this system was operated at a higher filtration rate of 15 $m^3/m^2/h$ and therefore needed high energy and chemical substances as coagulants. In another study, Hussain, *et al.* [10] studied disinfection of some microorganisms in water using graphite adsorbent coupled with electrochemical regeneration. Their results indicated that this system is effective in removing fungi (47-88%) but not successful in removing bacteria. In our previous studies, several AOPs were studied using pulsed high voltage [8] and RFICP in batch process [11,12] for disinfection of total coliforms and fecal coliforms. Compared to the disinfection efficiency of total coliforms and fecal coliforms, the disinfection efficiency of *Salmonella* Typhi was lower [8,11,12]. The differences disinfection efficiency due to type of applied voltage, applied frequency and type of microorganism. The frequency of the RFICP system is an important parameter for the disinfection of microorganisms in water. Further experiments could be conducted with an increased frequency in the RFICP system to increase the active species in the water and heighten removal efficiency with respect to *Salmonella* Typhi.

Disinfection of bacteria by RFICP systems generally follows the first-order kinetics as explained by Eq. (2) as follows [8,10]:

$$\frac{dN}{dt} = -kN \tag{2}$$

where t is retention time (h), N is number of *Salmonella* Typhi at t (CFU/mL), N_0 is initial number of *Salmonella* Typhi (CFU/mL), and k is death rate (1/h). Based on the experimental data, the death rate constant of *Salmonella* Typhi was calculated by plotting of ln C/C_0 vs. t with Eq. (3) as follows:

$$\ln\frac{N}{N_o} = kt \tag{3}$$

The death rate constant falls in the range of 0.57-0.33/h (R^2 = 0.89~0.94) for the number of *Salmonella* Typhi from 28-44 CFU/mL. Regarding the run in series 2, the death rate constant was in the range of 0.33-0.18/h (R^2 = 0.89 ~ 0.96) for a flowrate increase from 5-20 mL/minute.

A decrease of the death rate constant when the flowrate was increased is consistent with the results of the removal of total coliforms and fecal coliforms using the RFICP system [12]. Purnell, *et al.* [5] monitored the behavior of phages and viral pathogens in a full-scale membrane bioreactor (MBR) based water-recycling system. Their results indicated that the combination of chlorination and MBR technology appears to have significant potential in removing bacteria (fecal coliforms and enterococci) but is not effective in removing viral pathogens (adenovirus). The RFICP in our study is a new technology to protect human health without chemical disinfection, thus circumventing the production of toxic disinfection byproducts in drinking water such as THMs.

3.3 Disinfection yield and Energy Consumption of *Salmonella* Typhi

The disinfection efficiency of pathogenic bacteria is best illustrated by the disinfection yield, defined as the amount of microorganisms or deaths per unit of energy consumed in the process [10,14]. The disinfection yield of bacteria is calculated using Eq. (4) as follows:

$$Y = \frac{N_0 . v . DE}{100 . P . t} \tag{4}$$

where *Y* is disinfection yield (CFU/KWh), *v* is volume of water in the reactor (L), *P* is average power (KW), *DE* is disinfection efficiency (%) and *t* is retention time (h).

The energy consumption is calculated using Eq. (5) as follows:

$$W = \frac{V . I . t}{2v} \tag{5}$$

where *W* is energy consumption (KWh/L), *I* is current (A), *V* is voltage (volt), and *v* is volume of water in the reactor (L).

Active species produced in relation to the disinfection of pathogenic bacteria have been reported in [9]. The continuous attachment of active species onto the cell membrane causes damage to the cell wall and eventually cell death. Disinfection of active species such as ozone (O_3) and hydrogen peroxide (H_2O_2)

is conducted in drinking water treatment plants. Examining the effect of the initial number of *Salmonella* Typhi bacteria, it was found that the disinfection yield of *Salmonella* Typhi was less significantly affected by the initial number of pathogenic bacteria, as shown in Table 2. It is suggested that the number of produced active species such as $^{\bullet}OH$ (higher oxidation potential) is much higher than that of ozone (O_3) or hydrogen peroxide (H_2O_2). Besides preventing the production of active species, one of the advantages of RFICP is that it can also produce ultraviolet light and shock waves [1].

As displayed in Table 2, our results also indicate that the disinfection yield increased with the increase of the flowrate. The increase of the disinfection yield shows that more active species were produced in the water. However, further experiments are needed to analyze the amount of active species produced during experiments. It is well known that frequency [11,12] and flowrate (in the present study) are important in predicting disinfection performance against pathogenic bacteria in water. Similar results were also found for energy consumption, as shown in Table 2. Hazmi *et al.* [8] found that the disinfection yield of fecal coliforms and total coliforms using the PHV system was higher than when using the radio frequency plasma system. This result indicates that the disinfection yield and the energy consumption using the PHV system were also higher.

Table 2 Disinfection yield and energy consumption.

Series 1			
	Number of *Salmonella* (CFU/mL)	**Disinfection yield (CFU/KWh)**	**Energy consumption (KWh/L)**
	28	752	0.28
	40	799	0.28
	44	746	0.28
Series 2			
	Flowrate (mL/minute)	**Disinfection yield (CFU/KWh)**	**Energy consumption (KWh/L)**
	5	784	0.28
	10	1256	0.14
	20	1889	0.07

The most important result in this study is that the flowrate showed a significant increase of disinfection yield and energy consumption of *Salmonella* Typhi. Most drinking water treatment plants use surface water as raw water containing a high amount of colloidal particles. However, our earlier studies on removing total coliforms and fecal coliforms from river water using pulsed high voltage and RFICP systems showed that the disinfection efficiency of total coliforms and fecal coliforms varied from 25 to 100% [8,11,12], so the effect of colloidal particles on the RFICP system is a very interesting topic to study in the future.

4 Conclusions

The radio frequency induced couple plasma (RFICP) system is efficient for disinfection of pathogenic bacteria in water. The death rate constant of *Salmonella* Typhi and the disinfection efficiency decreased from 0.57 to 0.33/h and from 75 to 48%, respectively with the increase of the initial number of *Salmonella* Typhi bacteria. The flowrate showed a significant effect on disinfection of *Salmonella* Typhi. Compared with the flowrate, the initial number of *Salmonella* Typhi bacteria showed a less significant effect on death yield and energy consumption. The death yield increased from 784 to 1889 CFU/KWh and the energy consumption decreased from 0.28 to 0.07 KWh/L with the increase of the flowrate from 5 to 20 mL/minute. The most important outcome of this research is that the flowrate is an important parameter in predicting disinfection performance against pathogenic bacteria in water. Further research is necessary to evaluate disinfection performance with regards to ground water and river water to study the effect of colloidals in water.

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