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Inactivation of microorganisms in drinking water using combined treatment with ultraviolet and sodium persulfate

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ABSTRACT

This paper examines the combined UV irradiation and sodium persulfate treatment for disinfection of microorganisms in drinking water. By comparison, the effect of UV irradiation and sodium persulfate inactivating on *Bacillus subtilis* spores was applied through individual and combined treatment. *B. subtilis* spores were selected as the surrogate bacteria for these inactivation experiments. As comparison, first, the inactivating effect of UV/sodium persulfate on *Escherichia coli* was investigated. A 7.24 log reduction was achieved after 10 min exposure to 0.113 mW/cm^2 and 0.5 mM sodium persulfate. The results showed that sodium persulfate alone caused barely any inactivation effect on *B. subtilis* spores, where only 0. 98 log inactivation was achieved at 0.5 mM sodium persulfate during 10 min contact time. *B. subtilis* spores can be inactivated by UV irradiation to a certain extent, which reached 3.50 log at a UV dose of 68 mJ/cm^2. The synergistic disinfection of UV and sodium persulfate was proved to be the optimal combined treatment methods which reached a 4.30 log inactivation. Ultrastructural changes caused by UV/sodium persulfate process were observed by transmission electron microscopy which showed that the cell wall became blurred and the inner cytoplasm was indistinguishable, followed by a complete leakage of the cytoplasmic content.

Keywords: UV; Sodium persulfate; Combined disinfection; *Bacillus subtilis* spores; Inactivation effect

1. Introduction

With the advantages of high efficiency, extensive-use, easy operation and no secondary pollution, ultraviolet (UV) disinfection technology has been used commonly in drinking water treatment for many years. It has been listed as the most efficient disinfection process in drinking water treatment in America [1]. Studies have shown that *Escherichia coli* can be inactivated by UV irradiation [2–4] while some other microorganisms, such as virus or microbes with spores were more resistant to UV irradiation. *Bacillus subtilis* spores were commonly selected as a representative of highly-resistant microbes because of its high UV dose requirement [5–12]. *B. subtilis* spores are also considered to be a good surrogate for parasitic protozoans like *Cryptosporidium* and *Giardia*. Moreover, *B. subtilis* spores are often used for UV reactor validation in Europe. Chemical disinfectants can also be combined with Ultraviolet disinfection in order to reduce chemical doses, while maintaining a stabilizing inactivating effect in water treatment.

Advanced oxidation processes (AOPs) are characterized by the generation of radicals (such as $^{\bullet}OH$) with strong oxidizing ability, which was used in drinking water and wastewater treatment. AOPs is also applied to synergistic disinfections in drinking water treatment [12,13]. Sodium persulfate ($Na_2S_2O_8$, SP) can be activated by UV to produce sulfate radicals ($SO_4^{\bullet-}$), which has a high standard redox potential (E_0 = +2.01 V). Hybrid of UV and SP is chosen

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in this study for the strong oxidizing property to analyze the inactivation effect firstly towards *E. coli* and then the possibilities of individual and synergistic effects of UV irradiation and SP disinfection towards *B. subtilis* spores.

2. Materials and methods

2.1. Reagents

All reagents, supplied by Sinopharm Chemical Reagent Company Limited (Shanghai, China), were analytical reagent grade. Distilled water for analytical use was from Direct-Q3 (MilliPore, Boston, Massachusetts, USA). Reagents and materials used were sterilized by autoclaving (120°C, 20 min).

2.2. Experimental apparatus

The collimated beam apparatus was composed of a 40 W low-pressure (LP) mercury lamp (wavelength of 253.7 nm, Philips, The Netherlands). The stainless steel tube (h × b × l) = 112 × 112 × 1,200 mm) used in the experiments, is illustrated in Fig.1. The monochromatic UV radiation emitting by this lamp was directed to the surface of the test samples. Various UV irradiances were obtained by adjusting the distance between the sample and the lamp. By virtue of a UV-M radiometer (Beijing Normal University Experiment Co., China), the average irradiance at the solution surface was measured [14], namely, 113.0, 56.5 and 28.3 μW/cm^2, respectively.

2.2.1. E. coli culturing and enumeration

E. coli (ATCC 1.3373) (China General Microbiolgical Culture Collection Center, Beijing, China) was obtained freeze-dried and rehydrated aseptically with Nutrient Broth (Sinopharm Chemical Reagent Company Limited, Shanghai, China). Broth cultures were incubated in a shaker at 37°C for 24 h. The cultures were purified by centrifugation (6,000 r/min, 10 min) and resuspended in physiological salt solution. After disinfection, samples were serially diluted and plated onto nutrient agar medium to enumerate [15].

2.2.2. B. subtilis spores culturing and enumeration

B. subtilis spores (ATCC 9372) (China General Microbiolgical Culture Collection Center, Beijing, China) were obtained freeze-dried and rehydrated aseptically with Nutrient Broth (Sinopharm Chemical Reagent Company Limited, Shanghai, China). Broth cultures were incubated in a shaker at 31°C for 48 h. The cultures were heated at 80°C for 10 min, purified by centrifugation at 6,000 rpm, for 10 min and resuspended in 0.9% physiological salt solution. After disinfection, the spores were serially diluted and plated onto nutrient agar medium to enumerate [15].

2.3. Methods

2.3.1. Experimental methods

Petri dishes (90 mm diameter) containing 40 mL samples were exposed to the UV and stirred gently on the magnetic stirrer. Sodium persulfate of various concentrations were prepared for specific samples. All disinfection processes were terminated by addition of 10% sodium thiosulfate ($Na_2S_2O_3$) and the samples were then serially diluted. Bacterium suspension (1 mL) after dilution were injected onto nutrient agar medium, and incubated with nutrient agar medium (37°C, 24 h) to enumerate the spores, using a pour plate method. All experimental steps were carried out in an aseptic manipulation room to prevent interference. Samples for the transmission electron microscopy (TEM) with and without treatment were prepared [15] and examined using a JEM-1230 TEM (JEOL, Tokyo, Japan).

2.3.2. Data presentation

The inactivation effect was evaluated through the inactivation level of *B. subtilis* spores, described as Eq. (1):

$$\log\left(N / N_0\right) = -kt \tag{1}$$

Where N (CFU/mL) and N_0 (CFU/mL) are the microbial concentrations after and before the disinfection process, k (min^{-1}) refers to the pseudo-first-order rate constant and t (min) is the contact time.

3. Results and discussion

3.1. Inactivation effect of UV and SP on E. coli individually and combined

Fig. 2a shows the inactivation effect of different UV irradiances intensity on *E. coli*. The response curves proved that *E. coli* could be efficiently inactivated by UV radiation, which is consistent with previous studies [2–4]. Over a 6.0 log reduction was reached after a UV dose of 68 mJ/cm^2.

However, sodium persulfate alone can hardly have any influence on the inactivation of *E. coli*, since only a 0.24 log inactivation was observed even under the optimal condition according to Fig. 2b. This phenomenon might arise from

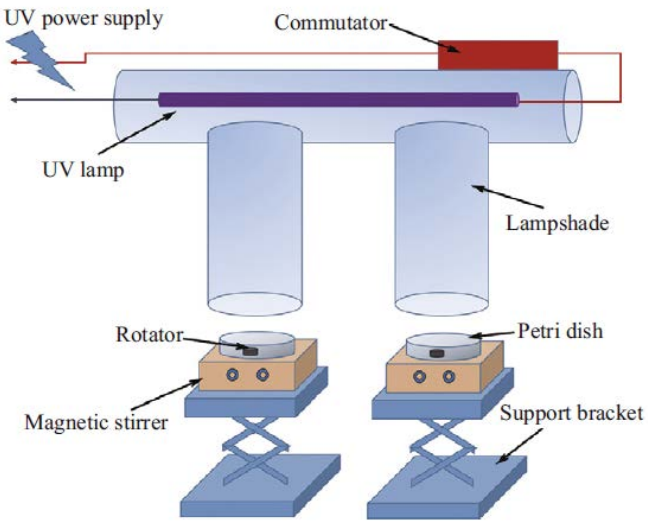

Fig. 1. Schematic diagram of collimated beam apparatus.

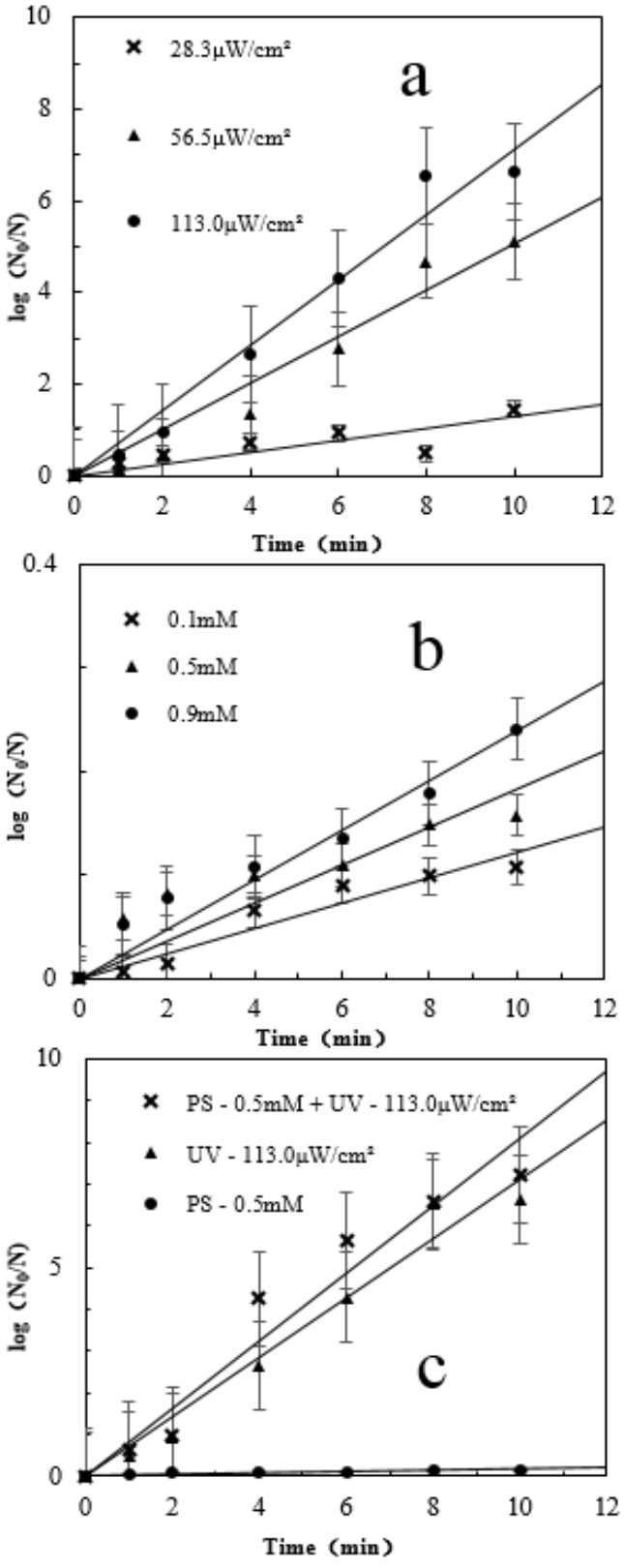

Fig. 2. Inactivation effect of SP and UV towards *E. coli*: (a) different UV irradiance intensities, (b) different SP concentrations, (c) comparation of simultaneous and individual of UV and SP (0.5 mM SP and 113.0 μW/cm² UV irradiance).

the fact that sodium persulfate is quite stable in aqueous solution at room temperature [16].

Fig. 2c compares the inactivation effect of UV and SP combined and individual on *E. coli* with 0.5 mM SP and UV dose 68 mJ/cm². The curves indicate that combined treatment of UV/SP has synergetic inactivation effects towards *E. coli*, about 7.0 log inactivation was achieved after a UV dose of 68 mJ/cm², which is higher than the effects of separate SP and UV treatments. Similar results have been achieved as seen as previous researches. Michael-Kordatou et al. [17] also founded the combined treatment of UV/SP in removing *E. coli* showed a synergistical effect by the addition of SP. During this process, $SO_4^{\bullet-}$ radicals (and subsequently •OH depending on solution pH) was generated by UV_{254} activation, which can contribute to the inactivation of *E. coli*. Compared to that observed in the presence of the oxidant, the removal rate of UV treatment alone was significantly lower. It was concluded that simultaneous UV/PS is a viable option for *E. coli* disinfection.

3.2. Inactivation effect of UV on B. subtilis spores

Significant inactivation effect on *B. subtilis* spores was observed by UV irradiation in Fig. 3 after 4 min and about 3.50 log inactivation was achieved at an applied UV dose of 68 mJ/cm², which is 3.1 log lower than that of *E. coli* under the same conditions. This decrease may arise from the characteristic of *B. subtilis* spores with its special spore structure. Spores are a dormant form of bacteria caused by an adverse environment when cells do not metabolize. In the dormant stage, cells have a strong resistance towards radiation, temperature, pH and other bad conditions due to its dense cell membrane, so *B. subtilis* spores have a strong resistance to ultraviolet exposure [18], and spores cannot be inactivated as effectively as bacterial cells [19]. This can be easily seen in Fig. 3 compared to Fig. 2a. Higher UV dose favors higher inactivation, which coincides with the results of previous researches [20,21].

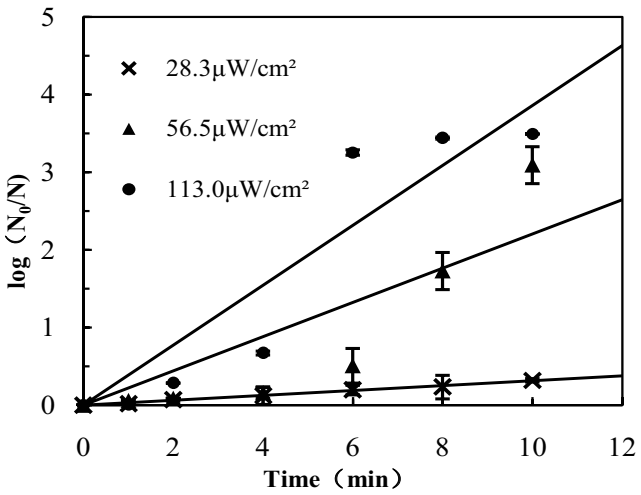

Fig. 3. Effects of different UV intensity and irradiation time on the inactivation rate of *B. subtilis* spores. UV irradiances intensity was 113.0.0, 56.5 and 28.3 μW/cm², respectively.

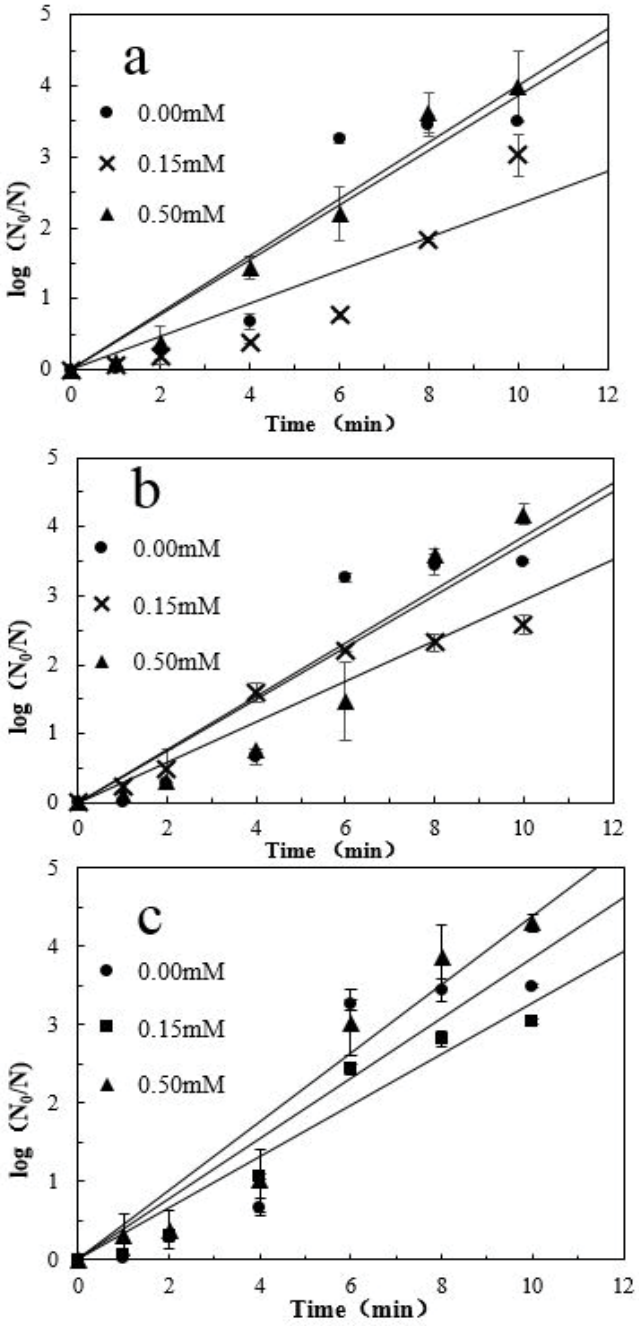

Fig. 4. Inactivation effect of different hybrid method of UV and SP on *B. subtilis* spores. (a) SP-UV, (b) UV-SP and (c) UV/SP. UV irradiances were 113.0 μW/cm² and SP concentration were 0, 0.15 and 0.50 mM respectively. Error bars represent the standard deviations.

3.3. Inactivation effect of SP on B. subtilis spores

To investigate the SP inactivation effect on *B. subtilis* spores, the SP concentration was applied as 0.1, 0.3, 0.5, 0.7 and 0.9 mM respectively. However, under the highest concentration, only a 0.98 log inactivation could be reached.

As seen in the Tables 1 and 2, the ideal results cannot be achieved by SP alone. Although SP ionizes to peroxodisulfate ($S_2O_8^{2-}$) in water, which has similar standard redox potential as ozone (peroxodisulfate $S_2O_8^{2-}$ standard oxidation–reduction potential E_0 = +2.01 V, ozone E_0 = +2.07 V), SP is relatively stable at room temperature, thus the inactivation effect is not obvious [16].

Table 1
Inactivation effect on *B. subtilis* spores alone in the dark for 10 min at different SP concentration

SP concentration (mM)	Inactivation (log)
0.1	0.613
0.3	0.612
0.5	0.977
0.7	0.582
0.9	0.610

Table 2
Inactivation effect of contact time on *B. subtilis* spores at 0.5 mM SP

Contact time (min)	Inactivation effect (log)
1	0.246
2	0.665
4	0.723
6	0.749
8	0.955
10	0.977

3.4. Inactivation effect of combined UV and SP on B. subtilis spores

The inactivation effect of three combined UV/SP treatments (UV exposure followed by SP treatment), SP-UV (PS treatment followed by UV exposure) and UV/SP (Simultaneous UV and SP) was compared at a 113.0 μW/cm² UV irradiance. As illustrated in Fig. 6a–c, SP was spiked into the system, which cannot always lead to an inactivation augment. It was assumed that in order to dissociate peroxodisulfate ($S_2O_8^{2-}$) in water, a certain amount of UV was consumed rather than only contributed to inactivation. When the peroxodisulfate was acquired in the initial phase, the inactivation effect would suffer a setback. After SP reaches certain concentration, it can bring enough effect to balance the UV loss. The three combined methods can improve the inactivation effect, so the treatment results without SP (0.00 mM) are always range between 0.15 mM and 0.50 mM. Taking SP-UV for instance, the inactivation almost reaches 4.0 log at 0.50 mM SP after a UV dose of 68 mJ/cm², namely, it was 0.50 log higher than by UV alone. Furthermore, it obtained 0.67 log by UV-SP. Combined UV and SP is superior to the other two methods by remarkably increasing the inactivation to 4.30 log after 10 mins under SP concentration of 0.50 mM.

On the basis of Fig. 4a–c, it was found that combined UV and SP disinfection can efficiently inactivate *B. subtilis* spores especially when they existed simultaneously. Previous researchers have investigated the synergistic

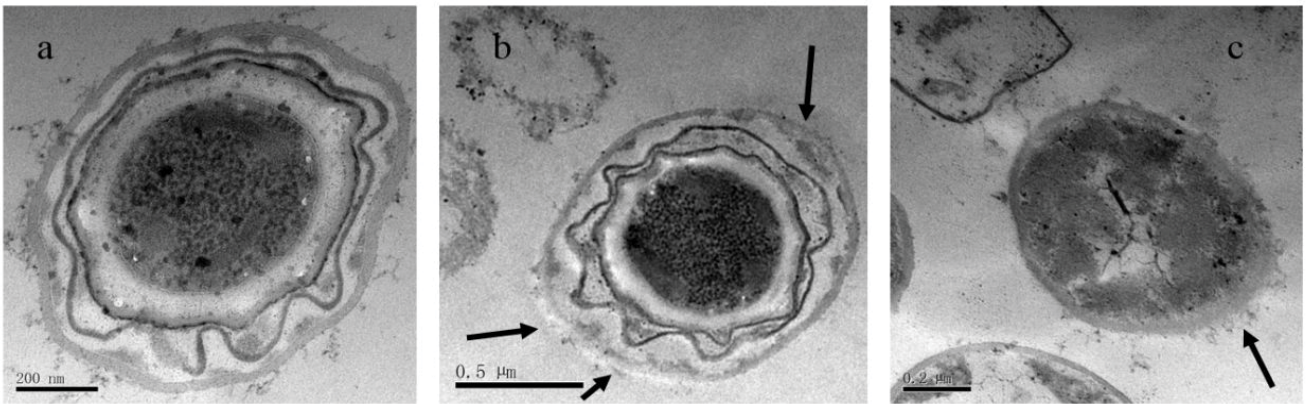

Fig. 5. TEM images of (a) untreated, (b) UV-treated and (c) UV-SP-treated *B. subtilis* spores (UV dose of 68 mJ/cm^2 and SP concentration of 0.5 mM).

mechanisms by combined disinfection, indicating that different disinfectants may act on distinct part of the cell, which increased the sensitivity of microorganisms to disinfectant, and thus applying two kinds of disinfectants at the same time may improve the inactivation effect [22,23]. Meanwhile the inactivation under UV irradiation depends very little on the type of spores, which is not the case when relying on chemically reactive species because of the varying composition of the spore coats that they have to cross before inactivating them [24]. These views further support the result that combined disinfection is optimal.

3.5. TEM analysis

In order to find the ultrastructural change caused by SP and UV irradiation, cell morphology images with and without disinfection were taken by TEM.

Fig. 5a shows an original untreated *B. subtilis* spore's cell with its intact cell structure. The cell is a round shape and has a spore coat, cell membrane and other cytoplasm. After it was exposed to UV irradiation (UV dose of 68 mJ/cm^2), the spore shell became partially indistinct as illustrated in Fig. 5b. Meanwhile, the other part of the cell still remains a relative integrity.

However, with the UV-SP treatment, it was observed that the cell was totally destroyed with the addition of 0.50 mM SP at a UV dose of 68 mJ/cm^2 from Fig. 5c. The outer layer became blurred and the inner cytoplasm was indistinguishable, followed by a complete leakage of the cytoplasmic content. Researchers (Grinshpun et al. [25], Cerf [26], and Broadwater et al. [27]) once suggested that it was necessary to reach a minimal level of damage in the spores coat before inactivation occurs. UV irradiation aims at the DNA of the microbes [28]. Through the TEM images, one can see the process of the inactivation, and the solid coat has to be damaged to a certain extent to lead to the decline of the live cells. Obviously when the sulfate radical and the UV irradiation act together on the cell coat, this could certainly shorten the time of its decomposition [19], UV could destroy the DNA even much easier. Based on the above analysis, it was concluded that the mechanism of the inactivation by simultaneous UV and SP treatment was the destruction of the solid spore coat. Besides, the effect of UV/SP disinfection were much better than that of UV or SP, respectively.

4. Conclusions

UV treatment alone has a certain inactivation effect on *B. subtilis* spores, and the performance was better at higher irradiation intensity, but SP alone could hardly achieve the ideal effect.

The efficiency can be improved by using combined UV and SP, and simultaneous UV and SP was the optimal method at a certain concentrations range of SP. On basis of the TEM image analysis, the decomposition of the cell wall of *B. subtilis* spores resulted in cell inactivation.

Acknowledgements

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