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Antibacterial activities of mechanochemically synthesized perovskite strontium titanate ferrite metal oxide

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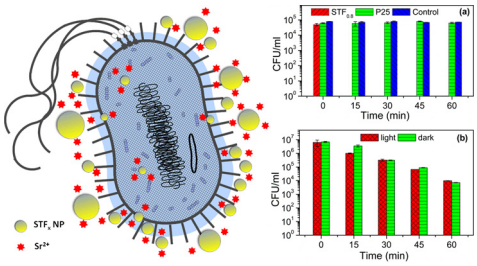
HIGHLIGHTS

- Perovskite STF_x was synthesized by mechanochemical high-energy ball milling.
- $STF_{0.8}$ shows excellent bactericidal effect in both light and dark conditions.
- Bactericidal mechanism is investigated to account for its excellent performance.
- Surface charge, high pH, Sr^{2+} and nano-size appear to be the key contributors.

GRAPHICAL ABSTRACT



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ABSTRACT

This work explored strontium titanate ferrite ($SrTi_{1-x}Fe_xO_{3-\delta}$ or STF_x in short) metal oxide as an effective antibacterial agent and investigated its bactericidal mechanism. The perovskite STF_x nanoparticles ($x = 0$, 0.2, 0.4, 0.6, 0.8 and 1) were successfully synthesized with high energy ball milling approach. The feasibility of utilizing this material for antibacterial application was studied on *Escherichia coli* (*E. coli*) in the presence of dispersed $STF_{0.8}$ nanoparticles in water. Excellent bactericidal effect has been observed by killing all the *E. coli* ($\sim 10^5$ CFU/mL) within 15 min in both light and dark conditions, excluding photocatalysis as the major contributing mean of bactericidal effect. Mechanism study via surface charge characterization, fluorescence microscope observation, inductively coupled plasma measurement and SEM examination has revealed that the positive surface charge, high pH environment, Sr^{2+} dissociation and nano-size of $STF_{0.8}$ metal oxide could have collectively contributed to its excellent bactericidal effect. These results have increased the potential to apply STF_x in water purification for microorganism destruction.

1. Introduction

With the positive bactericidal effect of TiO_2 photocatalyst reported in 1985 by Matsunaga [1], the destruction of microorganisms with photocatalytic technology has been extensively studied [2,3]. Since the photocatalysis of many wide bandgap

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semiconductors including TiO_2 and ZnO is limited to UV light excitation, much research efforts have been devoted to the the search for alternative photocatalysts with high efficiency in the visible light range for better utilization of the solar energy or the relatively weak indoor lighting [4–8]. Among the various materials, the novel composite of strontium titanium ferrite ($SrTi_{1-x}Fe_xO_{3-\delta}$ or STF_x) has attracted our attention. It is a continuous solid solution between the two end members, strontium titanate ($SrTiO_3$ or STO) and strontium ferrite ($SrFeO_{3-\delta}$ or SFO) [9]. Rothschild has proposed that the bandgap energy of STF_x system follows the second order polynomial $E_g(x) = 3.26 - 1.93x + 0.54x^2$ eV [10]. Therefore, with increasing Fe content, the bandgap energy of STF_x reduces from 3.2 eV for STO to that of 1.8 eV for SFO, corresponding to a wavelength range from 387 nm to 689 nm, covering the visible light range very well. STF_x appears as a promising visible-light driven photocatalyst by coupling the photocatalytic property of STO [11] and small bandgap of SFO [12].

In literature, STF_x metal oxide has been synthesized via various approaches including high temperature solid state reaction [12], liquid mixed technique [13], and wet chemical routine [14] for vast applications in gas sensor [10], hydrocarbon sensor [15], fuel cell [16], and oxygen separation membranes [17]. As compared to these conventional synthesis approaches, mechanochemical process commonly used in metal alloying may offer some unique advantages in synthesizing STF_x. It is a ball milling process where a powder mixture placed in the bowl is subjected to high-energy collision from the grinding [18]. Thus, nanoparticles (NPs) can be obtained with large specific surface area and high reactivity. In addition, its room temperature ambient helps to eliminate the difficulties in oxygen stoichiometric control in STF_x by avoiding the usage of thermal energy which normally incurs loss in volatile components during synthesis [19].

In the present work, the STF_x metal oxide ($x = 0$, 0.2, 0.4, 0.6, 0.8 and 1) was synthesized by mechanochemical high-energy ball milling process. The feasibility of utilizing this material for antibacterial applications was evaluated for the first time. $STF_{0.8}$ was studied for the destruction of *Escherichia coli* (*E. coli*), an extensively studied Gram-negative bacterium favored for its rapid growth rate and simple nutritional requirements. The bactericidal mechanism was also investigated through various characterization tools.

2. Experimental

2.1. Material preparation

The STF_x metal oxide with different nominal composition ($x = 0$, 0.2, 0.4, 0.6, 0.8 and 1) were synthesized by mechanochemical approach from commercially available SrO (Aldrich, 99.9%), TiO_2 (Alfa Aesar, 99+%, rutile) and Fe_2O_3 (Alfa Aesar, 99.945%). These starting materials in stoichiometric proportion were first homogenized in an agate motor for 15 min. The mixture was then sent for high energy ball milling in air at room temperature using the Fritsch Pulverisette 5-planetary-ball milling system. The tungsten carbide (93 wt% WC and 6 wt% Co) vials (volume of 250 mL) and balls (inner diameters of 10 mm and 20 mm) with relatively high density of 14.75 g/cm^3 were used as milling medium to increase the impact energy. The weight ratio of ball-to-STF_x powder was set as 20:1. The milling speed was set at 200 rpm for 120 h with every 25 min of milling followed by 5 min of pause to prevent overheating of the milling system. After synthesis, the formed metal oxide was ground again in agate mortar for 15 min and kept for storage in sealed bottles. The commercial P25 TiO_2 nanoparticles (≥99.5%, Evonik) as a standard photocatalyst were used as received without further purification.

2.2. Antibacterial test

For preliminary study, the bactericidal effect of synthesized STF_x powders was first evaluated in the water suspension of $STF_{0.8}$ on the waterborne pathogenic microorganisms *Escherichia coli* (*E. coli*). The *E. coli* was modified from the basic *E. coli* strain MG1655 (ATCC 700926) by introducing the R6K-based suicide plasmid pCCS167 (resistance to chloramphenicol, *B. subtilis* gene encoding the enzyme levansucrase which confers lethality to bacterial cells when grown in the presence of sucrose, pDM4 derivative, carrying $P_{A1/04/03}$-*gfp*mut3* flanked by sequences corresponding to base-pairs 312037–312754 and 312771–313495 of *E. coli* MG1655 genome) [20]. The $P_{A1/04/03}$-*gfp*mut3* carried by pCCS167 work as a constitutive promoter driving the expression of green fluorescent protein gene, so that the *E. coli* can be observed under fluorescence microscope without addition of exogenous reagents [20,21]. Single-species planktonic cultures of the *E. coli* cells were inoculated in fresh Luria-Bertani (LB) medium and incubated at 37 °C with shaking at 250 rpm. After overnight cultivation, the bacterial culture was serially diluted with distilled water to reach an initial cell density of ca. 10^7 CFU/mL, where the optical density at 600 nm (OD_{600}) was measured to be 0.02 using a UV spectrometer (BioSpec-Mini, Shimadzu). 100 μl of the diluted *E. coli* was pipetted into 20 mL continuously stirred photocatalyst suspension (1 g/L) to start the test in a custom-built photocatalytic reactor equipped with 40 W office fluorescent light and yellow filters (GG435, Melles Griot) to cut off the UV-light portion for visible light activity study. The initial *E. coli* cell density is thus around 5×10^4 CFU/mL and the resultant light intensity shinning onto the suspension is around 1800 lx. At different time points, 100 μL of suspension from each sample was pipetted and underwent successive serial dilution and 100 μL of each dilution was spread onto the nutrient agar plates in triplicates. These agar plates were incubated lid down, at 37 °C overnight. The bactericidal effect is evaluated with the standard plate-count technique to calculate the number of viable cells in terms of colony-forming units. Control tests in the absence of any photocatalyst or with the TiO_2 P25 or in the dark condition were performed concurrently for comparative study following the same viability assay.

2.3. Analysis

The crystallographic structures of the synthesized STF_x metal oxide were studied using the X-ray diffractometer (D5005, Simens) with an X-ray source of 1.54 Å Cu Kα operating at 40 kV and 40 mA. Diffuse reflectance spectra were recorded in the range of 240–800 nm in reference to barium sulphate ($BaSO_4$) using a UV–vis spectrometer (UV-2450, Shimadzu) fitted with a multipurpose sample compartment (MPC-2200, Shimadzu). A Kubelka–Munk (KM) transformation was done on the reflectance data to obtain the absorbance $F(R)$ in UV–vis light region. The bandgap energy was estimated by extrapolating the linear part of the $(F(R)E)^{1/2}$ vs E Tauc plot to the photon energy abscissa, assuming indirect band gap transition [22]. For bactericidal mechanism study, surface potential and size distribution of the bacteria and powders were measured using the zeta sizer (ZEN3600, Malvern) after ultrasonic dispersion. The morphology of STF_x metal oxide coated on wafer was inspected using a scanning electron microscopy (SEM, JSM-7600F, JEOL). The interaction between *E. coli* and the metal oxide was observed using an Eclipse 90i inverted confocal laser scanning microscopy (Nikon) equipped with 488 nm and 543 nm lasers. The pH of the STF_x suspension was read with a portable pH meter (UB-5, Denver Instrument). The dissociation of metal ions in the STF_x suspension in water was monitored by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES,