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Bacteria inactivation using spinel cobalt ferrite catalyst

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The *E. coli* inactivation using hydrogen peroxide (H_2O_2) and cobalt ferrite granulated catalyst was investigated in a fixed-bed flow reactor. The $CoFe_2O_4$ catalyst was synthesized by the co-precipitation method, granulated, and annealed at 1150°C. X-ray diffraction analysis was used to identify the crystal structure of the catalyst. $CoFe_2O_4$ catalyst demonstrates good catalytic activity for bacteria inactivation in the presence of H_2O_2. An increase in the hydrogen peroxide concentration increases the inactivation efficiency. The reactor demonstrates the *E. coli* inactivation of 99.94% at the H_2O_2 hydrogen peroxide concentration of 15 mM and initial bacterial concentration of $6 \cdot 10^3$ CFU/L. The water disinfection using a fixed-bed reactor demonstrates the broad prospects for industrial use.

Keywords: fixed-bed reactor; *E. coli*; cobalt ferrite; hydrogen peroxide; bacteria inactivation.

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Introduction

Clean water is becoming less available to people [1]. Contamination of water with chemical and microbiological pollutants prompts people to solve this problem, namely to find new effective methods of cleaning and disinfection. Chemical pollutants are quite dangerous, but microbiological ones, which include bacteria and viruses, pose a greater danger due to their rapid spread among humans. That is why it is important to use innovative and highly effective methods for wastewater disinfection, which contains a large number of various pathogenic microorganisms.

Advanced oxidation processes are promising for polluted water disinfection [2,3]. Reactive oxygen species effectively neutralize the microorganisms that can harm people [2,4]. Fenton systems, as demonstrated by recent studies, are more effective, ecological, and economical compared to ozonation, UV irradiation, electrochemical oxidation, and sonolysis [5,6]. The catalyst plays an important role in the Fenton processes. It should be non-toxic, and effectively decompose used oxidants into free radicals (•OH, •OOH), which are responsible for the pathogens' inactivation. The most common option for hydroxyl and hydroperoxyl radicals generating is the decomposition of hydrogen peroxide [1,7]. Iron oxides and spinel ferrites have met these requirements [6,8]. For example, cobalt ferrite has been tested in real wastewater and found to be excellent in peroxymonosulfate (PMS) activation and *Escherichia coli* and *Enterococcus sp* bacteria disinfection [1].

The hydroxyl radicals formed during the catalytic decomposition of H_2O_2 possess a strong oxidizing effect, are toxic for biomolecules, and, therefore, easily damage the cell walls of microbes, organelles, and DNA. In addition, these radicals show a much higher reaction rate than chlorine, ozone, or potassium permanganate [5,9]. The effect of hydrogen peroxide on bacteria inactivation is evidenced by many studies [2,10–12]. L. Fernández et al. [2] inactivated *E. coli* bacteria using magnetite-based nanoparticles. It was shown that the inactivation percentage increased with increasing H_2O_2 concentration. Copper ferrite also actively generates hydroxyl radicals, which resulted in cell membrane damage and inactivation of *E. coli* (100 %) and *S. aureus* (96.4 %) bacteria [10].

I. de la Obra Jiménez et al. [8] have chosen iron oxides (hematite, magnetite) as heterogeneous catalysts for Fenton processes. The disinfection of municipal wastewater in reactors with natural catalysts was evaluated. The research was carried out at neutral pH, in the periodic and continuous flow modes. As a result, the authors obtained a complete inactivation of *E. coli* bacteria, namely a reduction of *E. coli* by 5-log. It is important to note that different types of inactivation mechanisms were observed in this case, but the heterogeneous Fenton process is the key. Therefore, water disinfection using the Fenton processes effectively solved the polluted water problems.

The complete wastewater treatment technology includes two processes: the degradation of organic toxicants and water disinfection. To date, there is a very small amount of research dedicated to *E. coli* inactivation using fixed-bed flow reactors. G.D.L.P. Vargas et al. [13] have shown that the rate of *Escherichia coli* inactivation increases with an increase in pH and H_2O_2 concentration. The presence of dissolved pollutants in domestic wastewater decreases the effectiveness of *E. coli* inactivation.

The key factors in evaluating whether a treatment technology is suitable for industrial use are cost and energy consumption. The paper [14] describes a microbubble-gas plasma reactor that produces a large amount of oxygen reactive species and nitrogen reactive species for the bacteria inactivation in sewage. The reactor reduces more than a 5-log of *E. coli* after 30 minutes at an energy consumption of 68 kJ/L. The addition of humic acid reduces the rate of *E. coli* inactivation: the inactivation of *E. coli* at the humic acid concentration of 0.0015% was less than 50% compared to the inactivation in pure water. L.Y. Ozer et al. [15] have used a fixed-bed reactor to investigate the antimicrobial properties of the nano-silver-4 wt.%-kaolin (nAg-4-Kn) composite deposited onto borosilicate glass beads (BGB). The tests were conducted in a fixed-bed reactor on effluent taken from a secondary sewage treatment plant. The results showed complete disinfection of the wastewater, which was maintained for several days. The nAg-4-Kn/BGB composites demonstrated high stability, which was confirmed by the composites' stability on the BGB surface. C. Pablos et al. [16] investigated the photocatalytic inactivation of *Escherichia coli* bacteria by titanium dioxide in a fixed-bed reactor. The efficiency of the reactor was studied and compared with the results obtained in batch mode. In both cases, photocatalytic inactivation was successfully achieved, for a 6-fold reduction in bacterial concentration. A fixed-bed reactor increases the rate of inactivation with increasing treatment time. It can be explained by the fact that the fixed bed reactor requires a longer irradiation time to completely inactivate the bacteria. It allows the use of a continuous process without the need for particle removal, making this immobilized system a good alternative for the successful scale-up of water disinfection technology. I. Thakur et al [17] have presented a low-cost Fe-TiO_2 composite for *E. coli* disinfection prepared by mixing clay with foundry sand and fly ash. The constant leaching of iron ions in acidic conditions into the solution led to the double effect of photocatalysis and photo-Fenton in the purification system. The composite beads were used 30 times and accompanied by a decrease in their photoactivity by only 11%. The corresponding results represent high prospects for dual technology for *E. coli* inactivation. The study [18] describes a reliable device for water disinfection using silver nanoparticles (AgNP), immobilized on the glass surface, in a continuous flow mode. It was found that the cleaning efficiency increases with an increase in the reactor contact time. A minimal release of silver (100 μg/L) into the treated water was observed throughout the test period.

Thus, the effectiveness of flow reactors depends on various factors such as reactor volume, catalyst nature, and treatment time. Bacterial inactivation is influenced by a range of different parameters: temperature, pH, and hydrogen peroxide concentration [19]. The peroxide technique has already demonstrated high efficiency in wastewater treatment due to the reactive oxygen species (ROS) formation [11]. Today, it is important to evaluate the suitability of the flow mode for industrial-scale use, maintaining the high efficiency of bacterial inactivation. It is assumed that the integration of flow reactors into the wastewater treatment system will ensure efficient and rapid treatment of effluents. Therefore, the aim of this work is to investigate a flow fixed-bed reactor filled with $CoFe_2O_4$ catalyst and H_2O_2 for water disinfection.

I. Experimental

1.1. Chemicals and microorganisms

$CoCl_2 \cdot 6H_2O$ (Carlo Erba reagents), $FeCl_3 \cdot 6H_2O$ (Carlo Erba reagents), NaOH, and hydrogen peroxide (31.5 %) were obtained from SferaSim (Ukraine). The model bacteria were Gram-negative, non-spore-forming bacteria *Escherichia coli (ATCC 35218)*.

1.2. Catalyst preparation and characterization

$CoFe_2O_4$ nanoparticles were synthesized by the co-precipitation method. The precise amounts of iron(III) chloride (27.05 g) and cobalt(II) chloride (11.91 g) were used as a source of Fe^{3+} and Co^{2+} cations, respectively. Metal salts were dissolved in 500 mL of water and the resulting solution was stirred for 30 minutes at 90°C. After that, a NaOH solution (5 M) was added dropwise as a precipitant to obtain a solution pH of 14. The mixture was further stirred with an overhead stirrer at 600 rpm for 30 min to complete the reaction. During the entire synthesis process, the solution was heated at 90°C in a water bath. The resulting black precipitate of $CoFe_2O_4$ was separated using a magnet, washed three times with distilled water, and dried at room temperature. The obtained $CoFe_2O_4$ powder was pressed into pellets, the pressing process is described in detail in our previous work [11]. Pellets with a diameter of 10 mm and a thickness of 3 mm were sintered for 6 hours at 1150 °C. The sintered tablets were crushed into granules with a size of 0.2-3 mm and filled into a flow-through reactor with an inner diameter of 21 mm and a length of 60 cm. The total mass of granules in the flow-through reactor was 445 g, and the filling volume was 130.5 mL.

The phase analysis of the catalyst was performed by X-ray diffraction analysis (XRD) using a Rigaku MiniFlex

600 diffractometer. The CuKα radiation at a wavelength of 1.5406 Å with the scan step of 0.02° (2θ) and the scan speed of 1°/min was applied.

1.3. Bacterial inactivation tests

Gram-negative *Escherichia coli* bacteria were plated on a sterile nutrient medium and cultivated for 24 hours at 37°C. The bacterial dilution was used to prepare the desired concentration of the bacterial solution. A bacterial suspension was prepared from the former colonies, serial dilutions were carried out, and 1 mL of the suspension was added to 1 L of distilled water. As a result, concentrations of 10^2 and 10^3 CFU/L were obtained. To inactivate *E. coli*, a series of hydrogen peroxide solutions with a concentration of 2.5, 5, 10, and 15 mM were used in the experiments. The initial bacterial solution and H_2O_2 solution were simultaneously fed into the reactor at a constant speed of 1.25 mL/min. To study the effect of temperature on *E. coli* inactivation, the experiments were performed at 20°C and 25°C. Residual bacteria were cultivated for 24 hours at 37°C. Endo's medium helps identify *E. coli* bacteria by forming red colonies. Colonies were counted and the inactivation percentage was calculated according to the formula:

$$\% \text{ inactivation} = [1 - N_{out}/N_{in}] \times 100\% \quad (1)$$

where N_{in} and N_{out} are the concentration of bacteria at the inlet and outlet of the reactor.

II. Results and discussion

2.1. X-ray analysis

X-ray diffractograms of as-prepared and calcined at 1150 °C $CoFe_2O_4$ samples are shown in Fig. 1. The diffractograms contain peaks with indices (111), (220), (311), (222), (400), (422), (511), (440), (533) and (622), which belong to the face-centered cubic crystal structure of spinel [20,21]. It can be seen that the rise in annealing temperature leads to significant peaks' narrowing. The narrow peaks of the $CoFe_2O_4$ (1150 °C) sample confirm the formation of particles with a high degree of crystallinity. The detected XRD peaks correspond to the space group Fd3m. The lattice parameters equal a = 8.3749 Å and a = 8.3628 Å for as-synthesized $CoFe_2O_4$ and $CoFe_2O_4$-1150°C, respectively. The crystallite size (D) was calculated by the Scherrer method using the formula: $D = k \cdot \lambda / (\beta_{1/2} \cdot \cos\theta)$, where k is the Scherrer constant that takes into account the shape of the particles (k = 0.94), λ is the X-ray wavelength (nm); $\beta_{1/2}$ is the width of the peak at its half maximum (in radians); θ is an angle for peak (311) (in radians). The calculated crystallite sizes of $CoFe_2O_4$ and $CoFe_2O_4$-1150°C samples are approximately 14 and 98 nm, respectively.

2.2. Inactivation of bacteria *E. coli* using the catalytic decomposition of H_2O_2

The catalytic activity of $CoFe_2O_4$ was studied on opportunistic bacteria *Escherichia coli*. The work focuses on water disinfection using a reactor filled with a spinel ferrite catalyst using low concentrations of peroxide, which will reduce the negative effects on human health [1,22]. It is known that the $CoFe_2O_4$ catalyst can effectively decompose H_2O_2 to aggressive free radicals. These reactive oxygen species, as researchers have shown, are the main reason for the destruction of bacteria, their cell walls, DNA, and internal organelles [16]. Figs. 2a-f show the main results of this study. The initial bacterial suspensions contained $7 \cdot 10^2$ and $6 \cdot 10^3$ CFU/L of *E. coli* bacteria. The concentration of hydrogen peroxide varied from 2.5 to 15 mM. Studies at room temperature showed a reduction of *E. coli* bacteria in water by 1.81-log and 1.96-log at a concentration of 15 mM H_2O_2 for $7 \cdot 10^2$ and $6 \cdot 10^3$ CFU/L *E. coli*, respectively (Fig. 2a-b). The inactivation percentage increases with increasing concentration of hydrogen peroxide and is equal to more than 98 % at 15 mM of H_2O_2 (Fig. 2e). Thus, the higher the concentration of H_2O_2, the greater the number of ROS generated on the catalyst's surface [16].

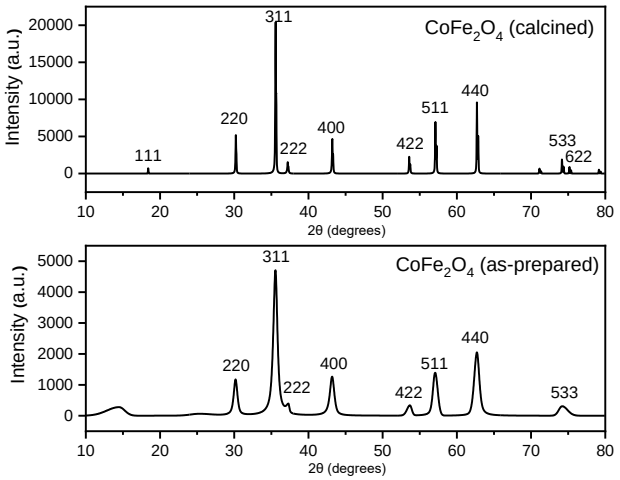

Fig. 1. XRD patterns for as-prepared and calcined at 1150 °C cobalt ferrites.

In addition, it was found that temperature change has a significant effect on bacteria inactivation. Conducting research on the catalytic activity of cobalt ferrite under conditions of elevated temperature, namely 25°C, is more favorable for *E. coli* inactivation (Fig. 2c-d). This is evidenced by the inactivation %, which is equal 99.89% at the initial concentration of *E. coli* $7 \cdot 10^2$ CFU/L and 99.94% at $6 \cdot 10^3$ CFU/L in the presence of 15 mM H_2O_2 (Fig. 2f). Presumably, as the temperature increases, the catalyst more actively decomposes H_2O_2 and generates a higher amount of hydroxyl and hydroperoxyl radicals, which inactivate gram-negative *E. coli* bacteria.

Fig. 3 shows the impact of experimental parameters (initial H_2O_2 concentration and temperature) on the *E. coli* inactivation. An increase in the H_2O_2 concentration and temperature from 20 to 25 °C causes a gradual increase in the inactivation effectiveness. The maximum efficiency of *E. coli* inactivation is observed at $[H_2O_2]_0$ = 15 mM and T = 25°C. The *E. coli* inactivation was mostly caused by hydrogen peroxide, which is a ROS source. It should be emphasized that the catalyst, prepared from sintered $CoFe_2O_4$ granules, requires rather low concentrations of H_2O_2 (10-15 mM) for the complete inactivation of *E. coli* bacteria.

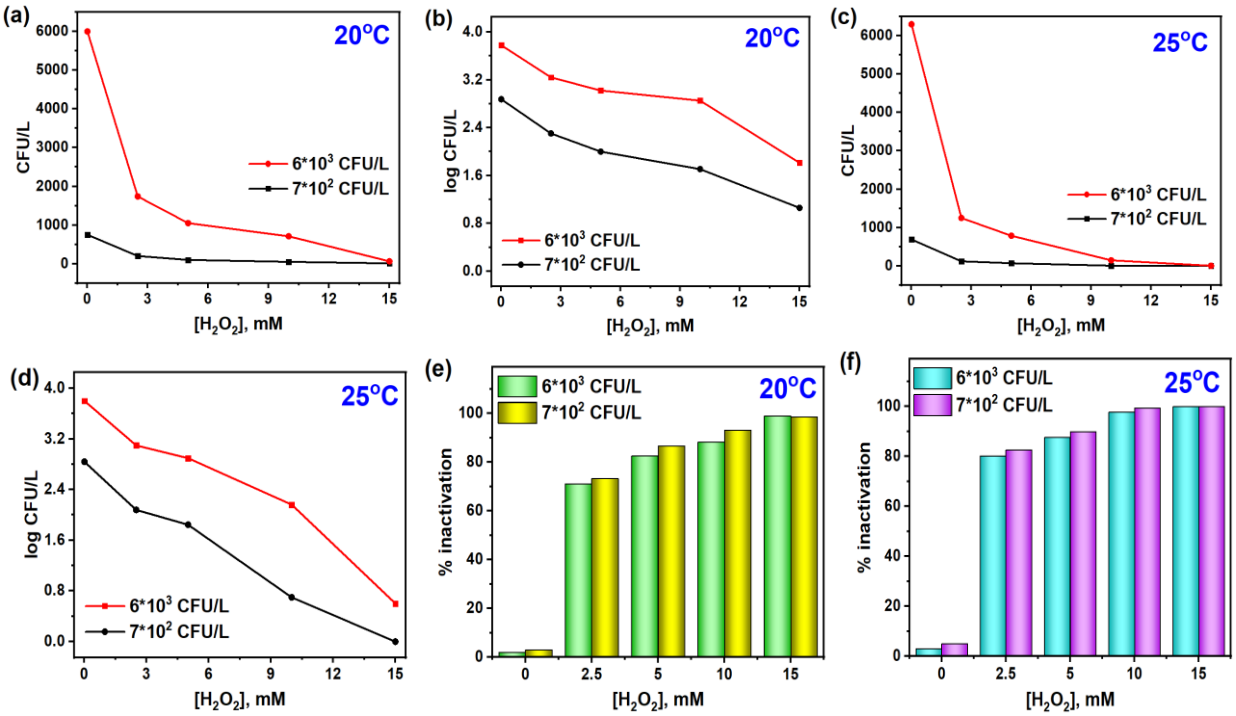

Fig. 2. (a) The *E. coli* bacteria inactivation at (a, c, e) 20 °C and (b, d, f) 25 °C: (a, c) bacteria count at the outlet of the reactor depending on the H_2O_2 concentration; (b, d) the log inactivation; (e, f) disinfection efficiency.

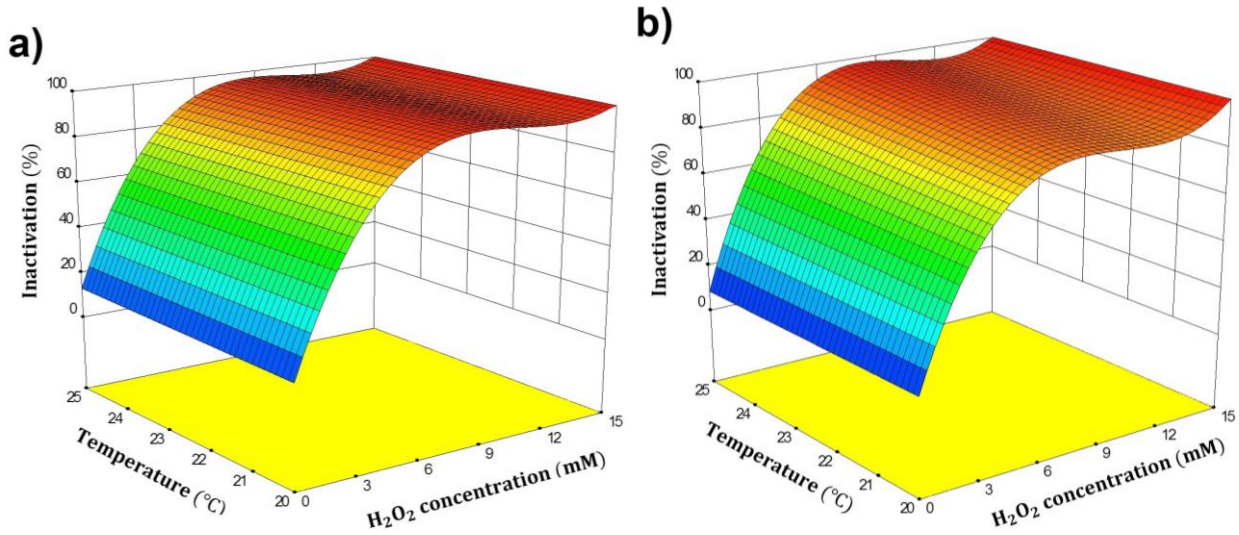

Fig. 3. (a, b) 3D graphs showing the impact of experimental parameters ($[H_2O_2]_0$ and T) on the *E. coli* inactivation: (a) $[E.\ coli]_0 = 7 \cdot 10^2$ CFU/L; (b) $[E.\ coli]_0 = 6 \cdot 10^3$ CFU/L.

Conclusions

Water disinfection using a flow fixed-bed reactor filled with $CoFe_2O_4$ granules showed good efficiency at an initial bacterial concentration of $7 \cdot 10^2$ and $6 \cdot 10^3$ CFU/L. The tests were performed at different hydrogen peroxide concentrations (0, 2.5, 5, 10, and 15 mM) and different temperatures (20°C and 25°C). The maximum efficiency of the flow reactor is observed using 15 mM H_2O_2: the inactivation of *E. coli* is more than 99 % at the initial *E. coli* concentration of $7 \cdot 10^2$ CFU/L and $6 \cdot 10^3$ CFU/L. The contact time of 52.5 min was sufficient for the *E. coli* inactivation. This approach can be used to treat industrial wastewater without adverse effects on the environment.

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Інактивація бактерій за допомогою шпінельного кобальт-феритного каталізатора

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В роботі досліджено інактивацію бактерій *E. coli* з використанням пероксиду водню (H_2O_2) і гранульованого каталізатора фериту кобальту в протічному реакторі з нерухомим шаром. Каталізатор $CoFe_2O_4$ синтезували методом співосадження, гранулювали та відпалювали при 1150°C. Для аналізу кристалічної структури каталізатора використовували Х-променевий аналіз. Каталізатор $CoFe_2O_4$ продемонстрував хорошу каталітичну активність щодо інактивації бактерій у присутності H_2O_2. Збільшення концентрації пероксиду водню підвищує ефективність інактивації. Реактор демонструє інактивацію бактерій *E. coli* на рівні 99.94% при концентрації пероксиду водню 15 мМ і початковій концентрації бактерій $6 \cdot 10^3$ КУО/л. Знезараження води за допомогою реактора з нерухомим шаром демонструє широкі перспективи для його промислового використання.

Ключові слова: реактор з нерухомим шаром; *E. coli*; кобальтовий ферит; пероксид водню; інактивація бактерій.