Removal of *Escherichia coli* from ballast water via high-gradient magnetic separation

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**Abstract.** Ship ballast water is a prominent medium for the growth of several foreign species. This study aimed to develop a high-gradient magnetic separation (HGMS)-based method to eliminate *Escherichia coli* and the mechanism underlying *E. coli* death, per regulation D-2 of the Ballast Water Convention. A novel wheat straw magnetic seed characterized by good flocculation performance, cost-effectiveness, and environmental friendliness was prepared via chemical implantation. The HGMS system using this wheat straw magnetic seed had a significantly higher total suspended solid (TSS) removal rate and *E. coli* removal rate than a system comprising Fe\(_3\)O\(_4\) (MP) + polyaluminium chloride (PAC) + polyacrylamide (PAM). A hydraulic retention time of 60 s, these rates were increased by 18.5% and 0.15 log, respectively. Furthermore, with an increase in magnetic field strength, the *E. coli* removal rate continued to increase, and *Escherichia coli* cells were markedly damaged. Lipid peroxide MDA levels increased rapidly over a short period, and a large volume of K\(^+\) leaked out, eventually leading to cell death. The present HGMS system using wheat straw magnetic seeds is a novel ballast water pretreatment system with good potential applications.

1. Introduction

With the continuous developments in society, the marine ecological environment is under unprecedented pressure [1-2]. Among various modes of pollution, ship ballast water discharge is a prominent cause of biological invasion, disruption of the marine ecological balance, and marine pollution [3-4]. The Ballast Water Convention was enforced on September 8, 2017, requiring all ocean-going vessels to be equipped with ballast water treatment systems [5]. The existing ballast water treatment methods mostly include a combination of two or more technologies, i.e., composite methods, wherein filtration technology is extensively used at the pretreatment stage of these composite methods [6]. However, ballast water entering the ship in offshore areas contains numerous flocs, which can easily block the filter screen, thus warranting frequent screening of the backwash filter. While this is time- and energy-consuming, it has no effect on microorganisms smaller than 20 μm. Some microalgae, bacteria, and viruses are difficult to eliminate via filtration [7-8].

In recent years, high-gradient magnetic separation (HGMS) has emerged as a water treatment technology, generally applied during pre-treatment filtration [9]. Compared with conventional filtration technologies, HGMS has advantages including large processing capacity, high efficiency, and simple
and compact equipment [10-11]. Previous studies have reported that the use of HGMS technology to treat urban sewage, supplementation of magnetic seeds and coagulants improved the removal rate of algae, bacteria, and other microorganisms up to more than 95%; organic matter, more than 85%; chroma and turbidity, more than 75%; these results indicate that HGMS technology can eliminate various pollutants simultaneously, with multifunctionality and versatility [12], thereby compensating for the limitations of conventional purification methods.

Magnetic media (Fe₃O₄, polyaluminium chloride, polyacrylamide [MP+PAC+PAM]) adopted for HGMS technology and magnetic field strength are important factors influencing the magnetic separation effect [13]. Most current studies focus on surface issues of the technology [14-15], while the selection, preparation of magnetic media, and the mechanism underlying the effects of magnetic field strength on microbes and their elimination via by magnetic separation technology play an important role in an ideal effluent outcome. Current studies suggest that disruption of tissue structure and metabolite levels of microorganisms via magnetic separation techniques are the two primary pathways causing microbial death [16-17]. However, further more precise studies are required for the verification of these experimental results, and the mechanism underlying microbial inactivation, e.g., bacteria, is yet unclear, presenting obstacles to its long-term effective operation.

Wheat straw (WS) is rich in natural polymers including cellulose and lignin, and has a numerous active groups such as hydroxyl groups and carboxyl groups distributed throughout its molecular chains, thus facilitating flocculation and complexation adsorption of water pollutants [18-19]. Based on these reports, this study aimed to develop a magnetic medium with good flocculation performance, strong removal ability, cost-effectiveness, and environmental friendliness. We developed an HGMS system comprising a novel type of wheat straw magnetic seeds (WSMS) and compared the removal effects of WSMS and MP+PAC+PAM on *Escherichia coli* and total suspended solid (TSSs) in seawater, examined the potential of WSMS to eliminate *E. coli* under different magnetic field strengths, and investigated the mechanism underlying *E. coli* death.

2. Materials and methods

2.1. Water sample preparation and bacterial strain culture
Simulated seawater was prepared using a Mocledon artificial seawater recipe with a salinity of 31±1‰, a temperature of 20±1°C, and pH 8.2±0.1. A suspension containing 200 mg L⁻¹ TSS was prepared using clay mineral.

As the International Maritime Organization uses *E. coli* as a bacterial indicator of human health in accordance with the G8 guidelines, *E. coli* was purchased from the China Center of Industrial Culture Collection and used in this experiment. *E. coli* was inoculated into sterilized LB liquid medium during the experiment and cultured in an electrothermal constant-temperature shaking tank (HZQ-X100A; Feiyue, China) at 37°C for 20 h for it to approach the logarithmic phase. Thereafter, the medium was centrifuged (TGL20M, Yingmin, China) at 4000 rpm for 10 min, and the supernatant was discarded. The precipitate was re-dissolved in seawater and formulated into a bacterial solution with a density of approximately 10⁷ CFU mL⁻¹.

2.2. Preparation and characterization of wheat straw magnetic seeds
To reduce the dosage of magnetic medium and further improve the efficiency of coagulation and sedimentation, chemical implantation was performed in this experiment [20]. WSMS was prepared by implanting Fe₃O₄ magnetic particles into non-magnetic WS. FeCl₃·6H₂O and FeSO₄·7H₂O at a mass ratio of 2:1 were dissolved in deionized water, followed by addition of pulverized WS powder. Under nitrogen protection and magnetic stirring (HJ-6; jieruier, China), 25% ammonium hydroxide was slowly dripped into this mixture, which was then allowed to stand in a water bath (HH-1; AHYQ, China) at 70°C for 4 h, followed by magnetic separation and drying at 60°C (DHG-9030; YIHENG, China) for further use.
The crystal structure of the wheat straw magnetic seeds was analyzed via a completely automatic X-ray powder diffractometer (XRD) (D8; BRUKERX, Germany). The surface morphology of wheat straw magnetic seeds was observed via scanning electron microscopy (SEM) (S-4800; HITACH, Japan) at a working voltage of 12.5 kV.

2.3. Experiment device
The experimental device used herein is shown in Fig. 1. A certain amount of magnetic medium was added to raw water, and raw water was thoroughly mixed with the magnetic medium. Under the action of the water pump, the water flew in from the lower inlet of the high-gradient magnetic filter and was intercepted by the magnetic field area. After treatment, the water flew out from the upper outlet. The experiment was carried out at 20°C and a water flow rate of 200–1200 Lh⁻¹, at a hydraulic retention time of the water flow reaching the sampling port of 10–60 s.

![Figure 1. A schematic representation of the high-gradient magnetic separation system.](image)

The removal effect of WSMS (150 mgL⁻¹) and MP + PAC + PAM (150 mgL⁻¹, 45mgL⁻¹ and 1.5 mgL⁻¹) on *Escherichia coli* and TSS in seawater under the same magnetic field strength (0.1T) was examined at different reaction durations. The effect of different magnetic field strengths (0.1, 0.3, 0.5, and 0.7 T) on elimination of *E. coli* by the HGMS system was investigated. Membrane damage in *E. coli* (formation of cell membrane lipid peroxide MDA and intracellular substance K⁺) and changes in the activity of intracellular superoxide dismutase (SOD) were assessed under different magnetic field strengths. All experiments were performed in triplicate, and error bars represent the standard deviation values.

2.4. Determination of the *E. coli* removal rate
*E. coli* contamination was determined using the membrane filter method [21]. After sampling, an appropriate dilution ratio was selected in accordance with the requirements, the sample was serially diluted by 10-fold, and the water sample was filtered through a 0.45-μm pore size filter membrane. Thereafter, the filter membrane was placed on mTEC medium and cultured at 35±1°C for 2 h. After incubation for up to 24 h at 44.5±0.5°C, *E. coli* formed red or purple-red colonies. The viable bacteria in the water sample were enumerated. The number of viable bacteria in the treated sample was compared with that in raw water to reflect the *E. coli* removal capacity of the treatment system, i.e., the *E. coli* removal rate (η), determined using the following formula:

\[ \eta = -\lg(N_f/N_0) \]
where $N_0$ - the number of viable bacteria in the sample before treatment, CFU mL$^{-1}$; $N_f$ - the number of viable bacteria in the sample after treatment for a certain period, CFU mL$^{-1}$.

2.5. Detection method

Malondialdehyde (MDA) and superoxide dismutase (SOD) levels in the samples were determined via the barbituric acid (TBA) method and pyrogallol method [22-23]. The $K^+$ concentration was determined via inductively coupled plasma atomic emission spectroscopy (ICP-AES).

3. Results and discussion

3.1. Characterization of wheat straw magnetic seeds

To observe the changes in the surface of WS powder before and after implantation of $\text{Fe}_3\text{O}_4$ magnetic particles, it was characterized via SEM. As shown in Fig. 2a, the surface of WS before implantation of $\text{Fe}_3\text{O}_4$ was relatively smooth because the WS surface had a very smooth cuticular wax film comprising high-grade aliphatic derivatives [24]. Simultaneously, the internal voids of WS were dense and had a large adsorption area. After the $\text{Fe}_3\text{O}_4$ magnetic particles were implanted into the WS, the surface of WS was rough and loaded with numerous $\text{Fe}_3\text{O}_4$ magnetic particles with uniform and fine distribution (micrograph in Fig. 2b), indicating that chemical implantation for surface modification of WS powder yields good loading effects of $\text{Fe}_3\text{O}_4$.

Figure 2c shows the XRD diffraction patterns of WS and WSMS. According to the JCPDS card No.19-0629 of the magnetite standard, XRD peaks at 30.2°, 35.5°, 43.2°, 53.4°, 57.3°, and 62.7° corresponded to the crystal planes (220), (311), (400), (422), (511), and (440) of $\text{Fe}_3\text{O}_4$, respectively. The diffraction angles ($2\theta$) of WS and WSMS were 12.24° and 13.38°, respectively. The WS and WSMS voids determined in accordance with the Bragg equation were 14.45 nm and 13.22 nm, respectively, indicating that $\text{Fe}_3\text{O}_4$ was successfully implanted into the wheat straw cellulose structure.

Figure 2. Scanning electron micrograph (SEM) and X-ray diffraction (XRD) images of wheat straw (WS) and WS magnetic seeds (WSMS) (a. SEM of WS; b. SEM image of WSMS; c. XRD pattern of WS and WSMS).

3.2. Effect of removal of different magnetic media on pollutants

This experiment was carried out at a TSS concentration of 200 mg L$^{-1}$, where the dose of WSMS was 150 mg L$^{-1}$, and the doses of MP, PAC, and PAM were 150 mg L$^{-1}$, 45 mg L$^{-1}$, and 1.5 mg L$^{-1}$, respectively. The effect of removal of different magnetic media on TSS is shown in Fig. 3a. At a hydraulic retention time of 10 s, the removal rates of TSS by WSMS and MP+PAC+PAM improved markedly, being 47.3% and 31.5%, respectively. However, on prolonging the hydraulic retention time, the increase in TSS removal rates by WSMS and MP+PAC+PAM were decelerated. At a hydraulic retention time of 60 s, the TSS removal rates by WSMS and MP+PAC+PAM were 75.7% and 57.2%, respectively. WSMS displayed better TSS removal effects on water quality than ordinary MP+PAC+PAM. The removal effect of different magnetic media on $E.\ coli$ is shown in Fig. 3b, similar to the results obtained with
TSS. At different hydraulic retention times, WSMS yielded better *E. coli* removal rates than MP+PAC+PAM. At a hydraulic retention time of 60 s, *E. coli* removal rates by WSMS and MP+PAC+PAM were 0.73log and 0.58log, respectively.

When treating high-TSS sewage with WSMS modified via Fe₃O₄ implantation, TSS and *E. coli* removal rates were significantly higher than those upon using MP+PAC+PAM. When the hydraulic retention time was 60 s, the removal rates increased by 18.5% and 0.15 log, respectively, probably being closely associated with the structure and flocculation mechanism of WSMS. WS is rich in natural polymers such as cellulose and lignin, which maintain its pre-set form in an aqueous solution and render it strongly stable. In addition, numerous hydroxyl groups are distributed along the molecular chains in WS. After the surface hydroxyl groups are coordinated, the hydroxyl groups complemented by their combined form attain a precipitation state and bind to and sweep other flocs to form coarser flocs during their precipitation [25]. Therefore, WSMS flocs have a higher degree of flocculation and a lower effluent TSS.

![Comparison of removal effects of different magnetic media on total suspended solids (a) and *Escherichia coli* (b).](image)

**Figure 3.** Comparison of removal effects of different magnetic media on total suspended solids (a) and *Escherichia coli* (b).

### 3.3. Removal effects of different magnetic field strengths on *E. coli*
Magnetic field strength is an important determinant of the effect of HGMS. At a WSMS dose of 150 mg L⁻¹, we examined the effect of magnetic field strength on magnetic separation. The results are shown in Fig. 4. When magnetic field strength increased from 0.01 T to 0.07 T, *E. coli* removal rates with different hydraulic retention times increased rapidly with an increase in magnetic field strength. However, when the magnetic field strength increased from 0.05 T to 0.07 T, the *E. coli* removal effect was not markedly augmented, probably because an increase in magnetic field strength increases the adsorption force on magnetic flocs, thus facilitating the capture of contaminants with weaker magnetism, thereby improving the separation effect. The difference in magnetism of the contaminants in the solution after WSMS coagulation was minor. Therefore, when the magnetic field strength approached a particular level, the magnetic force generated is sufficient to completely capture the magnetic flocs, and further increments in magnetic strength has limited effects on the *E. coli* removal rate. Furthermore, in practical engineering, an increase in magnetic field strength increases energy consumption and operational costs. Therefore, enhancing the magnetic separation effect is neither economical nor ideal, simultaneously presenting technological limitations. These results indicate that the optimal magnetic field strength for this purification method is 0.05 T.
Figure 4. Comparison of the removal effects of different magnetic field strengths on *Escherichia coli*.

3.4. Activity of intracellular MDA and SOD

Intracellular MDA levels can reflect the degree of cell membrane damage during the inactivation of *E. coli*. Changes in intracellular MDA levels in *E. coli* with time at different magnetic field strengths are shown in Fig. 5a. Under four magnetic field strengths, MDA levels in the bacterial suspension increased with an increase in hydraulic retention time. MDA levels at magnetic field strengths of 0.05 T and 0.07 T were significantly higher than those at magnetic field strengths of 0.01 T and 0.03 T, indicating that an increase in the magnetic field strength can cause more severe damage to the cells in a shorter period. During the sterilization process, harsher the cellular microenvironment, the easier it is to accumulate a large amount of active oxygen inside the cells, accompanied by the production of free radicals with higher activity, thereby causing the polyunsaturated fatty acids in the cells to undergo lipid peroxidation and form MDA. In addition, the resulting MDA concentration increases with an increase in sterilization time. However, when the magnetic field strength was increased from 0.05 T to 0.07 T, MDA levels in the bacterial suspension did not increase significantly, indicating that the optimal magnetic field strength is 0.05 T, consistent with the results of the analysis of the removal effect of different magnetic field strengths on *E. coli*.

Intracellular SOD level is a visual indicator of aging and death. To further investigate the mechanism underlying *E. coli* inactivation under different magnetic field strengths, changes in *E. coli* SOD activity with time are shown in Fig. 5b. At different magnetic field strengths, intracellular SOD activity of *E. coli* gradually decreased with an increase in hydraulic retention time. In particular, at magnetic field strengths of 0.05 T and 0.07 T, SOD activity decreased rapidly compared with that at magnetic field strengths of 0.01 T and 0.03 T, indicating that *E. coli* is stimulated by strong external oxidation reactions, and enzyme activity in *E. coli* is impaired by excess free oxygen radicals. Simultaneously, impaired enzyme activity can impair normal physiological functions of bacterial cells, and resulting in cell death.
Figure 5. Changes in intracellular malonaldehyde concentration (a) and superoxide dismutase activity (b) in Escherichia coli cells at different magnetic field strengths.

3.5. Analysis of leakage of intracellular material
On measuring the K⁺ content in the bacterial suspension at different magnetic field strengths, the leakage of intracellular material was assessed during sterilization, along with the degree of cell damage. The results of the experiment are shown in Fig. 6. Different magnetic field strengths can impair the permeability of the E. coli cell membrane. At relatively low magnetic field strengths, the cell membrane is slightly damaged, and K⁺ can penetrate the cell membrane and leak out of the cell. As K⁺ ions are small, they can leak out of the cell in large amounts after short-term sterilization. On extending the reaction time, bacterial cells were further damaged, and the leakage of K⁺ gradually increased. Combined with the results obtained on analyzing the E. coli removal effect at different magnetic field strengths (Fig. 4), impaired cell membrane permeability was the most prominent cause of cell death.

Figure 6. Changes in K⁺ concentration in Escherichia coli cells at different magnetic field strengths.

On examining the effects of different magnetic field strengths on cell membrane lipid peroxidation, changes in enzyme activity and leakage of cellular material of E. coli cells in seawater, microscopic examination revealed that the WSMS-based HGMS system has relatively good removal effect on microorganisms in ballast water. The present results provide strong theoretical support for the effectiveness and feasibility of this system in treating ship ballast water.
4. Conclusion
Currently, the Ballast Water Convention has been enforced. This study describes the development of a novel WSMS-based HGMS system with good flocculation performance, strong removal ability, cost-effectiveness, and environmental friendliness, generated via chemical implantation. This system displayed higher *E. coli* and TSS removal rates in raw water than the MP+PAC+PAM system. Moreover, with an increase in magnetic field strength, a large amount of active oxygen accumulated in the cells to generate free radicals with higher activity, thereby resulting lipid peroxidation of polyunsaturated fatty acids and formation of MDA. Simultaneously, *E. coli* enzymes were impaired owing to excess free oxygen radicals, thus impairing normal physiological functions in *E. coli*. Furthermore, a large amount of K+ leaked out of *E. coli* cells. These events eventually lead to cell death. The present results indicate that the WSMS-based HGMS system has potential for ballast water treatment.

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