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Research article

Improving adsorption effect of modified carbon felt on microorganisms in pig houses

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ABSTRACT

The pathogenic microorganisms in the air have a significant impact on piglet growth and even biosecurity of pig industry. Carbon felt-based microbial adsorption shows great potential in reducing the misuse of chemical disinfectants in pig houses. However, poor biocompatibility and low adsorption efficiency hinder the application of carbon felt for microbial control in animal husbandry. Herein, modified carbon felt was prepared with strong acid to improve its surface properties and internal structure. The hydrophilic and large specific surface area of modified sample offered high adsorption activity for bacteria adhered on biotic/abiotic interface. Fourier transform infrared spectrometer, X-ray diffraction, pore specific surface area analysis, and scanning electron microscopy were used to analyze the chemical functional groups and microporous structure of the modified carbon felt. Antibacterial tests were performed using the model bacteria *Escherichia coli*. Acid treatment converts the hydrophobicity of carbon felt to hydrophilicity, increasing adsorption capacity and promoting a disinfection rate of up to 97.3%. This study can enhance bioaffinity and adsorption selectivity of carbon felt to *Escherichia coli*, bringing its antibacterial activity and application prospects closer to industrialization.

1. Introduction

Today swine production has been industrialized all over the world, resulting in an increase in the use of confined buildings [1, 2, 3, 4, 5]. In swine confinement units, the high densities of animals can create airborne microorganism problems, which may directly impact the well-being of the animals [6, 7, 8, 9]. Compared with adult pigs with more resistant, piglets are more susceptible to airborne microorganisms in the environment [10]. Many airborne pathogenic microorganisms can cause diarrhea and even death in piglets, leading to serious economic losses to the pig farm. Examples include Porcine epidemic diarrhea virus, *Clostridium difficile*, *Streptococcus*, *Acinetobacter*, *Pseudomonas*, and *Escherichia coli* (*E. coli*) [11, 12, 13, 14, 15, 16]. In particular, *E. coli* considerably threatens piglet health due to its prevalence, direct pathogenicity, and high concentration in air environment. Therefore, the control of *E. coli* in the air environment of farrowing facilities has aroused widespread concern.

Chemical disinfection methods are commonly used in swine confinement buildings to effectively control *E. coli* [17]. However, chemical agents inevitably generate by-products, drug residues, and may increase drug resistance of pathogenic microorganisms [18, 19]. Alternatively, adsorption by which microorganisms are immobilized can be an efficient way to replace the chemical disinfection and solve the problem of *E. coli*. Carbon is widely known for its biocompatibility and absorbability [20, 21, 22], hence carbon-based materials may have a very broad development prospect in adhesion behavior of *E. coli*. Surface hydrophilicity and biocompatibility both have an impact on how effectively bacteria adhere to the surfaces of carbon materials. Bacterial adhesion is hindered by hydrophobic surfaces whereas it is considerably enhanced by hydrophilic ones. Large surface areas can also have a high capacity for adsorption, which promotes the adsorption of microorganisms. Kamitakahara et al. examined adherence of microorganisms on different materials, carbon coated material was more suitable for adhering microorganisms than poly vinyl chloride and polyurethane [23]. Also, a comparison of different materials showed that activated carbon fabric has the best adsorption properties for strong attachment of *Shewanella oneidensis* [24]. Chung et al. demonstrated that granule-activated carbon was excellent support for microorganism attachment for long-term experiment [25]. These studies inspired us to explore how the

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adsorption properties of carbon materials can be applied to control *E. coli* in swine confinement buildings.

In the pig industry, the running and playing behaviors of piglets can hinder the adsorption effect of carbon materials. *E. coli* could escape anytime and infect piglets with secondary bacterial infections [26]. In order to solve this problem, some studies have reported materials that directly kill microorganisms after adsorption. Huang et al. removed virus bioaerosol by using carbon nanotube corona discharge plasma technology and the removal efficiency of λ virus bioaerosols at discharge voltages of −7.5 kV was 91% [27]. The SiO_2–Ag nanoparticles was generated by Park et al., which included liquid droplets, was dried by a sheath airflow and directly deposited on the carbon materials to evaluate its anti-viral activity [28]. However, these technologies have a complicated procedure and a high cost, making them unfavorable for application in practical production. It is possible to improve adhesion by introducing functional groups to the surface. Not only can hydroxyl and carboxyl groups increase the hydrophilicity of surface, but they can introduce negative charges that attract bacteria and strengthen the bacteria that are already attached. Enhancing the adsorption of carbon materials to microorganisms by modification methods instead of killing microorganisms, and thus increasing the adhesion between the surface of the material and the cell can be the fundamental issue of the adsorption performance between pathogenic microorganisms and carbon materials.

In the present work, we developed a modified method of carbon materials for controlling *E. coli* in swine confinement buildings, and the effect was verified by simulation experiments. Carbon felt (CF) was selected as the adsorbent for *E. coli* in this study due to its good biocompatibility, large specific surface area and porosity, low cost, and ease of modification. For the application of modified CF, we tested not only its surface morphology and properties, but also the microorganism adhesion effect in simulation experiment. The corresponding removal efficiency of *E. coli* was significantly higher than that of unmodified CF. Our work can provide a scientific foundation for the application of carbon materials to improve the adsorption and adhesion performance for *E. coli*. We expect that this study can introduce a novel approach of microorganism control in swine confined buildings, and can be easily extended to other livestock and poultry breeding fields.

2. Materials and methods

2.1. Preparation of modified CF

Pretreatment of CF is necessary since commercial CF has rough surfaces with contaminants. CF was sonicated in acetone for 0.5 h to remove surface oil, then washed in an aqueous bath of 10% nitric acid solution at room temperature for 3 h to remove surface and interior ash. Blank CF was obtained after drying with hot air. The CF was then immersed in a mixture of concentrated sulfuric acid and concentrated nitric acid (v/v, 3:1) in a 60 °C aqueous bath for 2 h, whereupon CF was transferred to a newly produced mixed acid solution in an 80 °C aqueous bath for 0.5 h. Rinsed with a large amount of deionized water until the washing water was neutral, and dried in hot air to obtain A-CF.

2.2. Characterizations and facilities

The surface properties and adsorption performance of the modified materials were characterized. The surface hydrophilicity of CF and A-CF was obtained by a static water contact angle measuring instrument (DSA100S, KRUSS, Germany). The elemental composition and valence state of samples were performed by X-ray photoelectron spectrometer (XPS, ESCALAB 250Xi, Thermo Fisher, USA) under 0–1000 eV to analyze the chemical constituents of surface of the samples. The peak distribution and fitting of the XPS curve were performed using XPSPEAK Version 4.1. To obtain information of the crystal structure of carbon fiber, X-ray diffraction (XRD, D8-ADVANCE) was performed on the samples and the 2 theta was varied in the range of 10°–80°. Surface functional groups of CF and A-CF were characterized using Fourier transform infrared (FTIR, PerkinElmer, USA) over the range of 600–4000 cm^{-1}. Nitrogen adsorption isotherms measured at 77 K with ASAP 2010 instrument (Micromeritics Instrument Corporation, USA) were used to determine BET surface area and pore volume of the samples. The BET surface area was calculated according to the Brunauer-Emmett-Teller (BET) model. The total pore volume was obtained due to the nitrogen adsorption capacity when the relative pressure was 0.97. The data was processed and plotted by origin 2018."

2.3. Antibacterial activity simulation test

The airtight chamber was constructed with 1 m^3 cubic-shaped transparent acrylic panels to simulate a piglet nursery box. The CF or A-CF was fixed above the chamber, and the contaminated air containing *E. coli* was introduced into the chamber and blown by two small fans to keep the air uniformity. Contaminated air was obtained by purging *E. coli* colonies on solid LB medium and mixed with clean air to maintain a stable level of airborne bacteria. The level of *E. coli* chosen in this study was 3500 ± 100 CFU/m^3 based on the mean level of airborne bacteria in pig houses reported in the literature [26]. The number of *E. coli* in the air before and after CF or A-CF work was collected by natural precipitation method and counted with agar plate, and all samples were performed in triplicate [29]. After the simulation test, the morphology of *E. coli* adhesion on CF and A-CF was observed using a scanning electron microscope (SEM, Hitachi Regulus 8220, Japan) to analyze the material's adsorption effect. CF and A-CF were washed with sterilized PBS solution, immersed in 2.5% glutaraldehyde for 2 h, sequentially dehydrated with a series of ethanol solutions (30%, 50%, 70%, 90% and 100%) for 20 min, freeze-dried, and coated with a platinum layer for SEM imaging.

3. Results and discussion

3.1. Surface properties and composition

The waterdrop contact angle (WCA) test can be used to determine the interface contact angle by observing the morphology of static water droplets on the material's surface, thereby reflecting the bioaffinity of material. As shown in Figure 1a, CF and A-CF had a substantial disparity in WCAs of 108.5° and 34.0°, respectively. A WCA larger than 90° indicates that the surface of CF is more hydrophobic, which is unsuitable for microorganism adhesion [30]. The WCA of A-CF was an acute angle, indicating that the surface of A-CF was completely hydrophilic. The mixed acid treatment significantly improves the wettability of the surface of carbon felt, converting it from hydrophobic to hydrophilic. This is because the efficient treatment totally removes impurities and oil from the CF surface, and the strong oxidation of mixed acid effectively washes each carbon fiber, opening up the area inside the CF. To carbon fibers, the mixed acid may introduce uniformly dispersed hydrophilic groups, namely hydroxyl and carboxyl groups. The increase in hydrophilicity following mixed acid treatment reveals that A-CF has higher bioaffinity and provides a favorable environment for *E. coli* attachment, indicating that it might promote greater *E. coli* adsorption of A-CF.

The surface properties of two samples were analyzed using FTIR spectrograms in the wavelength range of 600–4000 cm^{-1}, which can assess samples by the relationship of transmittance or absorbance with wavelength. The FTIR spectra of CF and A-CF were compared in Figure 1b. The diffraction peaks at 1532 cm^{-1} and 1578 cm^{-1}, 1644 cm^{-1} and 1650 cm^{-1} in both curves were attributed to the stretching vibration of the C=C bond, which was likely related to the force between carbon atoms [31]. In contrast to the flat trend of CF around 3150 cm^{-1}, the broad peak formed by A-CF around 3150 cm^{-1} can be assigned to the stretching vibration of hydroxyl groups, while the spike formed by A-CF around 1024 cm^{-1} was assigned to carbonyl group [32]. This demonstrates that following mixed acid treatment, the hydroxyl and carboxyl groups are effectively modified on the surface of carbon felts. Since carboxyl groups also contain hydroxyl groups, the overall distribution of

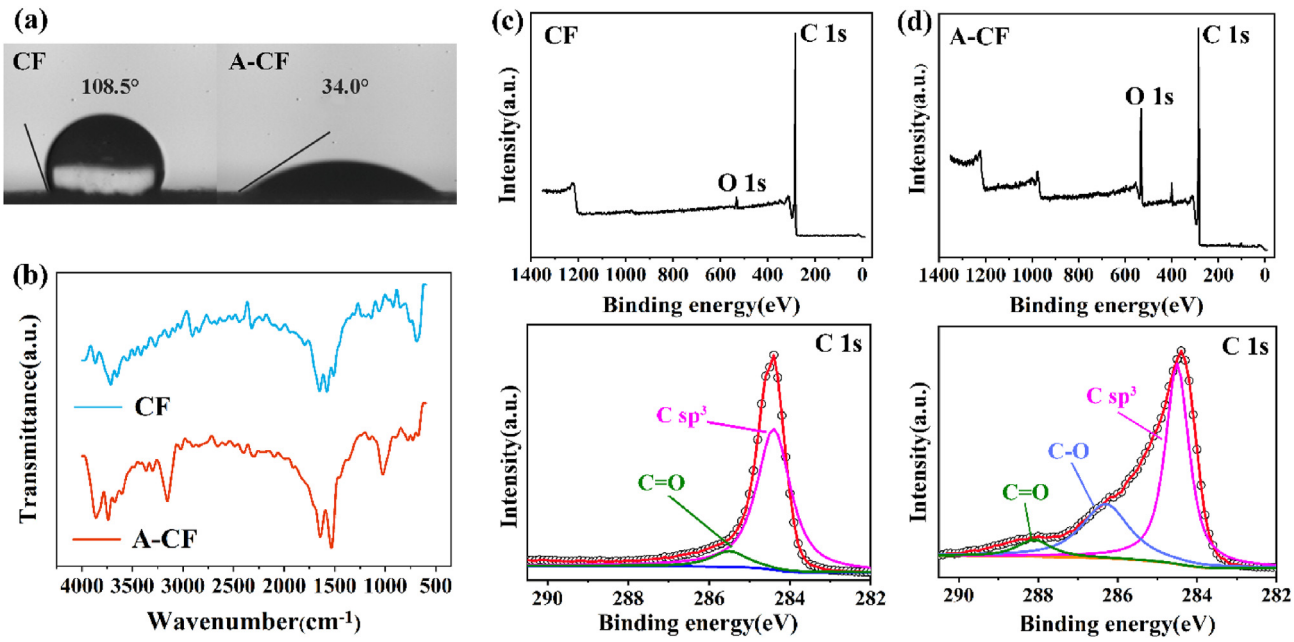

Figure 1. Extraction of the drop contour and contact angle measurement of CF and A-CF (a), FTIR spectra of CF and A-CF (b), XPS survey scan and the high-resolution C 1s XPS spectra of CF (c) and A-CF (d).

hydroxyl groups is denser than that of carboxyl groups, and the short distance between hydroxyl groups makes weak hydrogen bonds easier to form, resulting in wide peaks instead of diffuse spike bands. After mixed acid treatment, peaks corresponded to hydroxyl and carboxyl groups emerge in the FTIR spectrum, indicating that the surface of material has been effectively changed with functional groups of hydroxyl and carboxyl groups. This is also a major aspect in A-CF to dramatically increase its hydrophilic characteristics, hence increasing its bioaffinity, as seen by the results of WCA test. These hydrophilic groups can adsorb *E. coli* while also assisting in their adhesion, laying the foundation for the antibacterial function of A-CF.

Figure 1c and d showed that molecular composition and carbon bonding configuration of CF and A-CF were analyzed by XPS spectroscopy and high-resolution C 1s core level XPS spectrum. In the range of 0–1350 eV, both samples revealed the peaks of C 1s at 284.4 eV and O 1s at 532.4 eV [33]. The peaks were high and sharp because both samples were mostly composed of carbon. The O 1s peak of CF can be attributed to surface impurities or water, but O 1s of A-CF was attributed to the acidified carboxyl and hydroxyl groups, therefore the peak was higher and more visible. Further, the high-resolution C 1s core level spectrum was used to investigate the element composition of the two samples, and the corresponding chemical bonds were specifically analyzed by deconvolution peak. CF exhibited a C=O bond with a binding energy of 285.4 eV and the sp3 C atom at 284.4 eV, indicating the possible impurities on the surface and the carbon chain connection of the carbon fiber, respectively. The same peaks were separated in the A-CF spectrum, with the C=O bond (288.1 eV) assigned to the carboxyl group. The new peak of C–O bond at 286.3 eV was attributed to the hydroxyl group [34]. These XPS results are in good agreement with the reported hydroxyl and carboxyl groups, as well as with the FTIR spectra. These XPS findings accord well with the reported hydroxyl and carboxyl groups, as well as the FTIR spectra. It is demonstrated that CF is successfully modified, with hydrophilic carbon fibers forming on the surface and interior, promoting the adhesion and selectivity for *E. coli* on carbon felts.

3.2. Internal structure and adsorption capacity

The apparent structural information of CF and A-CF analyzed by XRD in the range of 0–40° was shown in Figure 2a. The spectra had a similar trend in comparison to the two samples, demonstrating that the acid treatment did not disrupt the basic structure of CF but preserved the interior porous structure. When considering carbon fibers as crystals, it can be found that the diffraction peak of A-CF was greater than that of CF at $2\theta = 25°$, which was due to the crystal size shrinking following acidification. It is consistent with the results reported by Mazarbhuiya et al. that the crystal lattice alteration of carbon materials by mixed acids [35]. The mixed acids roughen the surface of carbon fiber, allowing bacteria adhered more effectively. Acidification can thus improve CF adhesion and sterilizing efficiency, assuring the adsorption force.

The specific surface area test was performed to validate the adsorption capability of CF and A-CF. Figure 2b showed the adsorption isotherm of nitrogen on two samples. According to the IUPAC classification, these samples exhibited a typical Type IV isotherm with a type H3 hysteresis loop [36]. Obviously, the nitrogen adsorption capacity of A-CF was much higher than that of CF, indicating that A-CF has a stronger adsorption force. As shown in Table 1, after acidification, the specific surface area of A-CF obtained by BET analysis was 97.8 m^2/g, which was around 3.2 times larger than that of CF (30.2 m^2/g). In the middle pressure range, A-CF also featured a substantial desorption hysteresis loop. It demonstrates that A-CF has a more developed porous structure, which increases the sample's capacity and attractiveness to *E. coli*. After acidification, Pore volume and average pore diameter of CF increased from 0.176 cm^3/g, and 23.3 nm to 0.842 cm^3/g, and 34.4 nm, respectively. The slope of the low pressure range of the nitrogen adsorption isotherm can also reflect the specific surface area to some extent. It can be found that the slope of A-CF is larger than that of CF, implying that A-CF has a larger specific surface area. Combined with the results of XRD, the acidified samples have a superior porous structure and larger specific surface area, allowing for a greater capacity for *E. coli* adsorption. Because both the hydroxyl and carboxyl groups are charged, repulsive interactions may exist between carbon fibers, causing the internal space and pore structure to enlarge.

3.3. Antibacterial activity in simulation experiment

In order to better deploy carbon felt to piglet incubators, the operating parameters of the samples for *E. coli* adsorption were tested using a simulated scenario to verify the antibacterial activity of the samples against airborne bacteria. As the adsorbed item, *E. coli* is a typical model microorganism used in antibacterial experiments. As shown in Figure 3a,

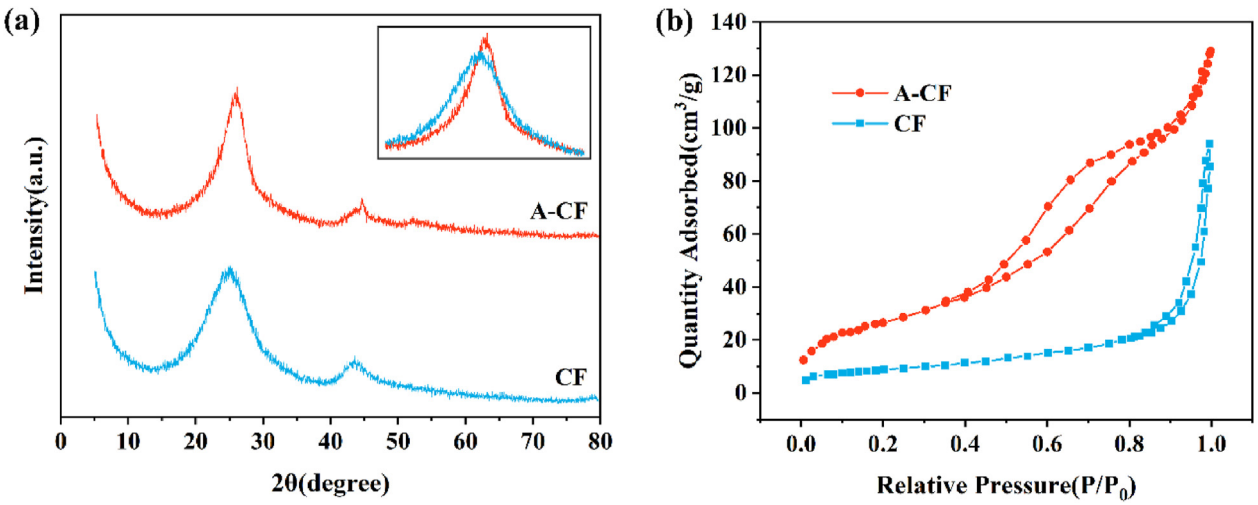

Figure 2. XRD patterns of CF and A-CF (a), and N_2 adsorption-desorption isotherms of CF and A-CF (b).

Table 1. Porous structure parameters according to BET analysis.

Sample	BET surface area/$m^2 \cdot g^{-1}$	Pore volume/$cm^3 \cdot g^{-1}$	Average pore diameter/nm
CF	30.2	0.176	23.3
A-CF	97.8	0.842	34.4

CF and A-CF with side lengths of 5 cm, 10 cm, 15 cm, and 20 cm, respectively, were performed in a 1 × 1 m airtight chamber within 60 min at the *E. coli* level of 3500 ± 100 CFU/m^3. It can be observed that in the presence of CF, the quantity of surviving *E. coli* continued to drop as the area grew larger, and the antibacterial effect was highest when the area of CF reached 20 × 20 cm (~290 CFU/m^3). Considering the expense of carbon felt fabrication, increasing the area of CF will make it more difficult to use in production. In general, the antibacterial activity of CF was lower than that of A-CF. The remained *E. coli* level was ~95 CFU/m^3 when the area of A-CF reached 10 × 10 cm, which means it was almost completely adsorbed. The antibacterial effect rose somewhat as the area of A-CF was enlarged, reaching a maximum of 98.9% (remained *E. coli* level was ~38 CFU/m^3). Although both samples had greater antibacterial effects as the area grew larger, it is obvious that A-CF of 10 × 10 cm had the most appropriate antibacterial activity (97.3%). Figure 3b showed that the number of *E. coli* adsorbed by 10 × 10 cm CF and A-CF varied over time. The decay trend of bacteria in the air corresponding to the two samples was first fast and then slow. After 80 min, the adsorption capacity of CF reached saturation (remained *E. coli* level was ~800 CFU/m^3), and its antibacterial activity began to decline. It's possible that the CF's attraction to *E. coli* weakened after 100 min, allowing some bacteria to escape and return to the air. During the same time period, adsorption capacity of A-CF was substantially higher than that of CF. It absorbed ~3400 CFU/m^3 of *E. coli* in 60 min and maintained that level to 120 min to prevent the bacteria from diffusion. It demonstrates that A-CF has a higher potential for adsorption and adherence to *E. coli* than CF. Some common chemical disinfectants with proven applications such as hypochlorous acid and ozone are highly efficient in sterilization [37]. Hao et al. studied the effectiveness of slightly acidic electrolytic water in chicken houses for disinfection of facility surfaces, with disinfection rate of 97% to bacteria and fungi at an effective chlorine concentration of 250 mg/L [38]. Joad et al. tested antibacterial activity of ozone with 100% of *E. coli*, 95.9% of *Staphylococcus aureus*, and 89.8% of *Pseudomonas aeruginosa* [39]. Using physical methods, Shen et al. reported Ag/zeolite as a filter material in an air purifier to remove microorganisms and the cumulative removal of bacteria and fungi after 24 h was 900, and 1088 CFU/g, respectively [40]. Modified CF had a higher bactericidal rate than Ag/zeolite and can be approximately as effective as those chemical disinfectants. According to the findings of the experiments, CF had a limited antibacterial activity, while A-CF showed 97.3% antibacterial performance at the size of 10 × 10 cm and running for 60 min. Under these

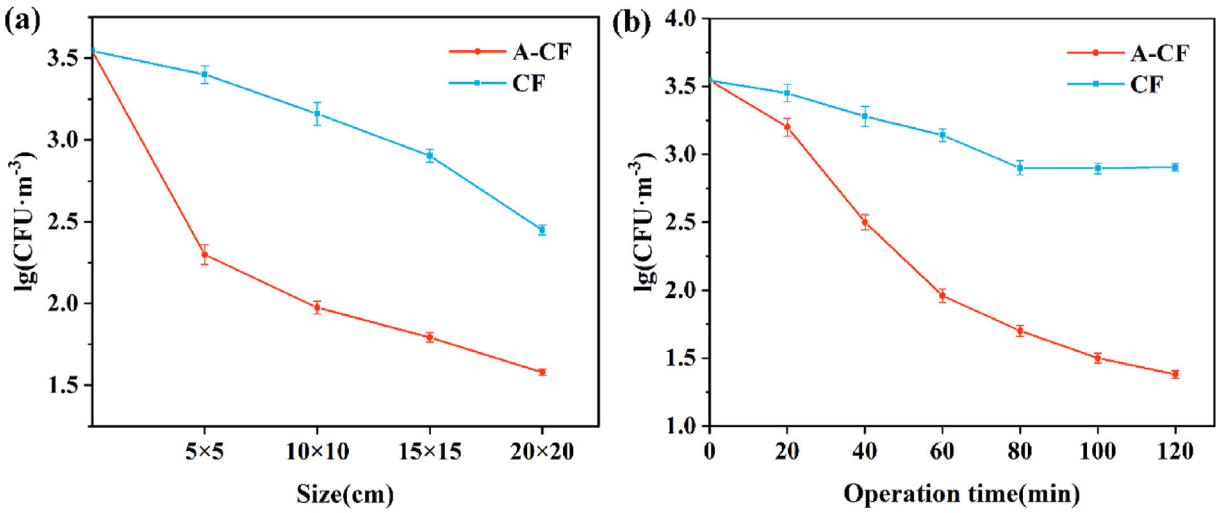

Figure 3. Antibacterial activities of CF and A-CF against *E. Coli.* with size (a) and operation time (b).

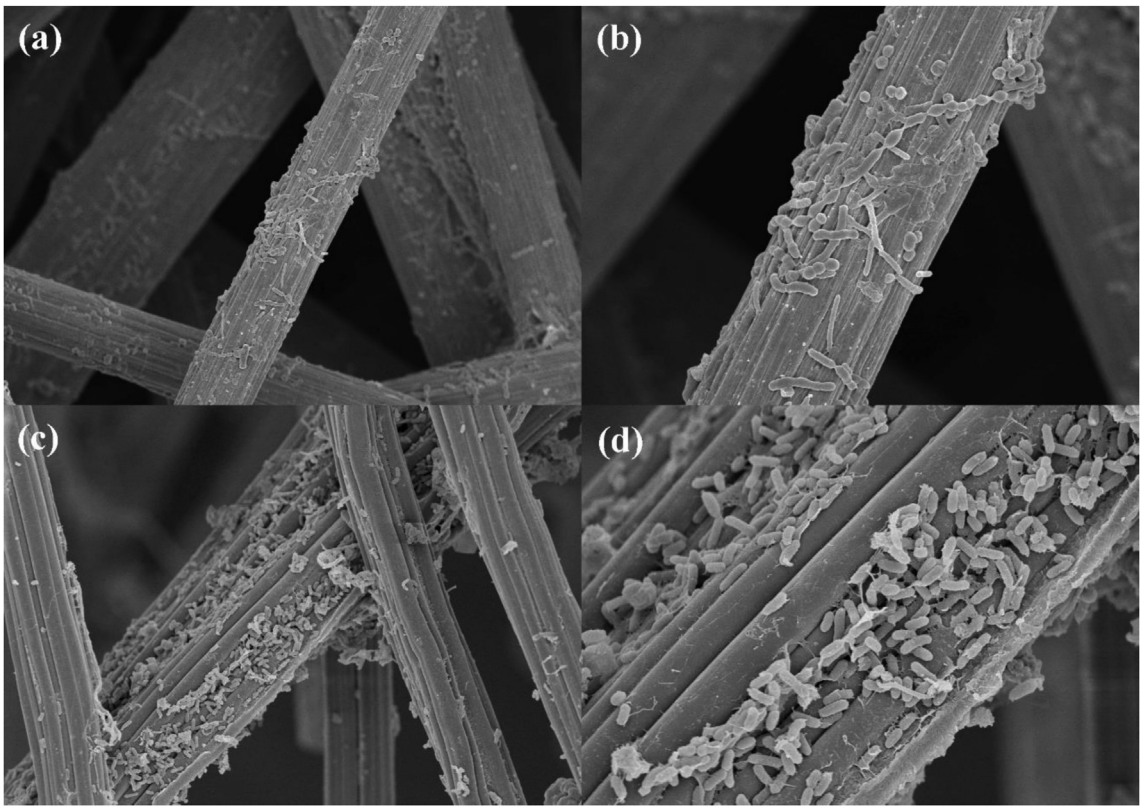

Figure 4. SEM images of CF (a and b) and A-CF (c and d) after *E. coli* adsorption at different magnifications.

conditions, A-CF can exert the maximum adsorption capacity, which is of great significance to the actual production of piglets.

3.4. Morphology of materials after operation

The surface morphology of CF and A-CF after the experiment was imaged by SEM to visually validate that A-CF had higher antibacterial activity. Figure 4 showed that the samples' internal spatial structure and the attachment of *E. coli* to them at different magnifications. The densely interwoven carbon fibers inside the carbon felt constituted a three-dimensional porous structure with a large specific surface area, which gave basic performance for *E. coli* adsorption. Bacteria attached sparsely and loosely to the surface of CF (Figure 4a and b), while bacteria adhering to A-CF were thicker and denser (Figure 4c and d). It was discovered that A-CF had a stronger adhesion property to *E. coli*, and that the acidified carbon fiber surface was more conducive to *E. coli* adhesion. The quantity of bacteria attached to A-CF was significantly higher than that to CF, which correlated to the level of antibacterial activity of the samples. In addition, the bacterial distribution of A-CF was more uniform, indicating that hydroxyl and carboxyl groups were introduced uniformly after acidification. To sum up, the acidified samples boost the hydrophilicity and bioaffinity of the surface, which can enhance the adhesion of bacteria on the carbon felt and further promote the interaction at the biotic/abiotic interface. The dense and uniform *E. coli* is adsorbed on the A-CF, demonstrating that the acidification procedure can indeed improve the adsorption capacity and antibacterial activity of carbon felt.

4. Conclusions

An innovative approach was provided to replace chemical disinfection in pig farms. Performance of CF can be increased by carrying out a facile operation technique. High adsorption efficiency combined with strong bioaffinity gave excellent control effect against *E. coli*. However, the actual application may be affected by unfavorable factors such as dust in the air environment of piggery, and the cost should to be further reduced for large-scale application.

In the present work, a facile acidification process was developed to modify carbon felt, and inhibitory impact on airborne microorganisms was demonstrated. The introduced hydroxyl and carboxyl groups increased the hydrophilicity of carbon fibers, which exhibited an excellent biological affinity for bacterial adhesion. The acidified samples retained the original three-dimensional porous structure while increasing the specific surface area, which promoted the increase of the adsorption capacity. The antibacterial test showed that 10×10 cm of A-CF working for 60 min could efficiently and economically control bacteria in the air, and the optimized sterilization rate was 97.3%. This work has provided a novel idea and showed broad prospects for controlling pathogenic microorganisms in the air, and we expect that our study can be further generalized in the biosecurity control of livestock and poultry industries.

Declarations

Author contribution statement

Xuedong Zhao: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Fei Qi: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Hao Li: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Zhengxiang Shi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

No additional information is available for this paper.

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