Effects of high-pressure activated slightly acidic electrolyzed water on cleaning and sterilization of pig transfer vehicles

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Abstract: The establishment of biosafety system is of enormous importance to the livestock and poultry production in terms of mitigating the transmission of diseases and implementing regional prevention and control measures. However, the current sterilization technology presents several drawbacks, including time-consuming procedures, chemical residues, and challenges in treating the sewage after rinsing. In this study, a novel cleaning and sterilization method that combines slightly acidic electrolyzed water and high pressure water-jet was developed. An orthogonal test was conducted to examine the correlation between high-pressure conditions and the various non-structural parameters on the efficacy of sterilization rate. In a field test, the effectiveness of the technology in cleaning pig transfer vehicles was evaluated by the total plate count and variations of community composition. The findings revealed that the combination of process parameters, including an available chlorine concentration of 200 mg/L, rinsing pressure of 170 bar, rinsing duration of 10 s, and residence time of 15 min, resulted in a removal rate of colony concentration on the surface of pig transfer vehicles of (96.50±0.91)%. Moreover, it was demonstrated to effectively inhibit a variety of pathogenic bacteria. The innovative cleaning system has the potential to replace traditional methods and reduces pollution while saving time and labor. It introduces a novel approach for sterilization of transportation in livestock and poultry farms as well as the biosafety construction of the animal husbandry.

Keywords: slightly acidic electrolyzed water, high pressure water-jet, sterilization, biosafety, concentrated animal production
DOI: 10.25165/j.ijabe.20241704.8847

Citation: Zhao X D, Tao S N, Qi F, Li H, Shi Z X. Effects of high-pressure activated slightly acidic electrolyzed water on cleaning and sterilization of pig transfer vehicles. Int J Agric & Biol Eng, 2024; 17(4): 53–59.

1 Introduction

The implementation of biosafety systems plays a crucial role in preventing the spread of diseases within the animal husbandry, particularly in densely concentrated and modernized livestock and poultry farms[1-3]. The sterilization method of various production processes in livestock and poultry farms is an important part of ensuring animal health and production. At present, the prevailing approach for sterilization involves the utilization of various disinfectants and detergents, which are applied repeatedly or for extended durations. The main components of typical disinfectants include strong acids and bases, potassium monopersulfate, trichloroisocyanuric acid, etc[4,5]. Prolonged usage of these can lead to the development of drug resistance, thereby potentially endangering the well-being of both individuals and animals[6,7]. Simultaneously, the efficacy of sterilization will be significantly reduced in the complex field environment, thereby generating a substantial quantity of chemical-laden sewage.

Slightly acidic electrolyzed water (SAEW) refers to an aqueous solution that possesses special physicochemical properties. It is generated through the process of electrolysis, wherein a dilute salt or acid solution is subjected to an applied DC electric field in a non-membrane electrolytic chamber[8,9]. It exhibits potent antimicrobial properties, effectively eliminating a diverse range of microorganisms and viruses such as *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*[10,11]. Many studies have demonstrated that the utilization of SAEW does not yield any discernible impact on animal health and production performance during the process of sterilization[12]. Hao et al. sprayed SAEW at an available chlorine concentration (ACC) of 300 mg/L to reduce 59% of the airborne organisms in 30 min and kept the population of microbes at a reduced level for at least 8 h in the pig house[13]. In the study conducted by Kawai et al., milking equipment and the parlor environment were sterilized with SAEW before and during milking time, and the incidence of clinical and subclinical *Pseudomonas mastitis* cases decreased significantly[14]. Ni et al. verified that SAEW with 80 or 100 mg/L of ACC significantly reduced microbial populations on each surface of the layer houses, especially *Staphylococci* and *Coliforms*[15]. However, the sterilizing effect of SAEW disinfection can be limited by the organic matter content[16], and pollutants containing substantial amounts of organic matter, such as feces, are the main source of bacteria and viruses in livestock and poultry farms. The efficient application of SAEW in the animal husbandry, as well as the establishment of innovative methods for its utilization, pose pressing challenges that require immediate attention.

Received date: 2024-02-02 **Accepted date:** 2024-06-05

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Traditional spraying, sprinkling and other sterilization operations have inherent limitations in thoroughly eliminating organic matter residue and various pathogenic bacteria. High pressure water-jet (HPWJ) technology is an efficient method for rinsing surface dirt by using high-velocity water flow[17], which creates an optimal environment for the disinfectant to carry out its intended function. Nevertheless, HPWJ has a wide range of applications and complex parameter settings. Medan et al. conducted a comprehensive investigation employing the full factorial approach to examine the impact of rinsing pressure, angle of incidence, and other parameters on the efficacy of HPWJ for cleaning purposes[18]. Cao et al. utilized CFD software to simulate the flow field both inside and outside the HPWJ nozzle, subsequently analyzing the jet axial velocity and pressure distribution[19]. It can be foreseen that by establishing suitable parameters, the technology of SAEW in conjunction with HPWJ can enhance rinsing and sterilization effectiveness, and show great potential to reduce sewage discharge and renovate sterilization method in livestock and poultry farms.

Herein, the combination of SAEW and HPWJ (SA-HP) technology was developed to boost the sterilization efficacy. Simulation tests were conducted to explore the impact of HPWJ technology parameters on the mechanism and effect of SAEW sterilization. Moreover, pig transport vehicles were selected as the application object in the field test, and the efficacy of the innovative technology was demonstrated through the observed alterations in total plate count and community structure. The objective of our research is to develop a novel and highly effective system of sterilization for animal production farms. Additionally, it is expected to provide a theoretical basis and technical support to enable livestock and poultry farms to reduce the potential risk of pollution and disease transmission in a sustainable manner.

2 Materials and methods

Simulation and field tests were conducted to determine the working parameters and methods of SA-HP technology for cleaning pig transfer vehicles. The simulation tests analyzed the optimal working parameters of SA-HP technology through the sterilization rate on scaled tires. The field tests verified the sterilization rate and community structure before and after operation on pig transfer vehicles with different cleaning methods. The overall research process is shown in Figure 1.

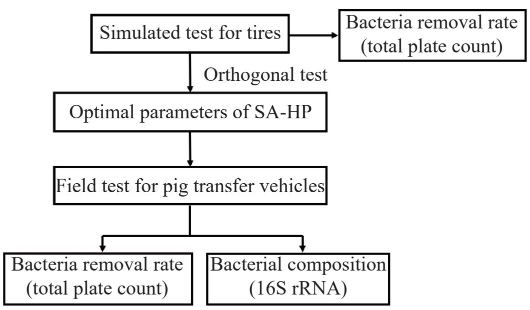


Figure 1 Overall workflow of the research process

2.1 Experimental apparatus

The experimental setup of SA-HP mainly consists of a water generation unit and a high-pressure rinsing unit. The primary component of the water generation system was a slightly acidic electrolyzed water generator (Beijing Rui'ande Environment Technology Co. Ltd., Beijing, China), which was prepared by electrolysis of 1 g/L NaCl solution, mixing the acid electrolyzed water (pH=2.8, ACC=18 mg/L) from the anode side and alkaline electrolyzed water from the cathode side. The final ACC in SAEW ranges from 80 to 250 mg/L, with a pH level between 5 and 6. The water production rate is approximately 100-250 L/h. The high-pressure rinsing system consists of a control unit, a high-pressure pump, a water demineralizing device, and a high-pressure water lance, and its protection level is IP55.

2.2 Design of simulation experiment

The effect of system parameters on the efficacy of sterilization was investigated in an animal environment simulation laboratory in Shangzhuang, Beijing, China. We utilized scaled-down tire models with a 1:10 isotropic ratio for conducting simulation experiments. In the single factor test, the ACC of SAEW was varied at levels of 50, 100, 150, 200, and 250 mg/L. The study of high-pressure system was subdivided into three parameters, namely pressure, rinsing duration, and residence time. According to the HPWJ operating within the range of 10-100 MPa, the injection angle was set to 90° and the rinsing jet distance was 0.25 m in order to maximize the striking force on the target surface. The experimental conditions consisted of four pressure levels: 110, 140, 170, and 200 bar. The time duration was varied across five different values: 5, 10, 15, 20, and 25 s. Additionally, the residence time was manipulated across five different intervals: 5, 10, 15, 20, and 25 min. Each set of tests was repeated three times. Further, based on the results of the single factor test, a three-factor, three-level orthogonal experimental design (Table 1) was employed to evaluate the optimal combinations of parameters for the process of rinsing and sterilization. The experiment was conducted with three replicates.

Table 1 Design of orthogonal test

Test number	Factor A ACC/mg·L^{-1}	Factor B Rinsing duration/s	Factor C Residence time/min
1	150	10	10
2	150	15	15
3	150	20	20
4	175	10	20
5	175	15	10
6	175	20	15
7	200	10	15
8	200	15	20
9	200	20	10

2.3 Field test

A field trial was conducted at a pig transfer vehicle decontamination center of pig farm located in Huanghua County, Hebei Province, China. The dimensions of the transport vehicles were measured to be 6.8 m in length, 2.4 m in width, and 2.6 m in height. They were divided into two groups, a control group and an experimental group, and each group consisted of 24 transfer vehicles. Before the test, both the control group and the experimental group were thoroughly cleaned of feces. Subsequently, the control group underwent a cleaning process using a foaming agent and disinfected by spraying with trichloroisocyanuric acid for 30 min. Based on the results of the simulated test, the experimental group was cleaned by high pressure rinsing using SAEW with an ACC of 200 mg/L and a pressure of 170 Bar for 30 min.

2.4 Detection and evaluation methods

The performance of SAEW was detected and controlled by pH, oxidation-reduction potential (ORP), and ACC. The ORP values of the SAEW were measured by employing a dual-scale meter

(Hangzhou Ying'ao Technology Co., Hangzhou, China). The ACC was determined using a digital chlorine test kit (RC-2Z, Kasahara Chemical Instruments Co., Saitama, Japan). The efficacy of sterilization was demonstrated through the alteration in the concentration of viable bacteria on the surface, as measured before and after the experimental procedure. In the simulation experiment, *Salmonella* (ATCC14028, purchased from the China General Microbiological Culture Collection Center) was used to make a contaminated tire because *Salmonella* is very common and pathogenic in pig farms[20]. After the tires were sterilized, the suspension of *Salmonella* was dropped by uniformly on the surface of tires to 7 lg CFU·cm^{-2} over a target area measuring 0.1×0.1 m. Samples collected from the tire surface after the test were incubated at 37°C for 24 h and counted to calculate the sterilization rate (Equation 1).

$$R = \frac{P_b - P_a}{P_b} \times 100\% \quad (1)$$

where, R is the sterilization rate; P_b is the total plate count before cleaning, CFU·cm^{-2}; P_a is the total plate count after cleaning, CFU·cm^{-2}.

Field tests were carried out utilizing sampling plates with a size of 0.1 m×0.1 m, which were placed in the designated sampling area. The outer surface of the vehicle and the inner compartment were uniformly sampled with 15 sampling points. Each sampling point was divided into two adjacent areas for sampling before and after cleaning, and was wiped with sterile gauze of normal saline for 25 times repeatedly. Each group of tests was repeated 3 times. These samples were subsequently stored at 4°C and transported to the laboratory for further analysis. One portion of the sample was incubated at 37°C for 48 h for total plate count of culturable bacteria. The remaining portions of the sample were analyzed for microbial diversity.

2.5 Community analysis

The total genomic DNA from the samples was extracted using the CTAB/SDS method, and the DNA's concentration and purity were assessed by DNA agarose gel electrophoresis. The samples were then melted, thoroughly mixed, and centrifuged before being subjected to electrophoresis on a 1% agarose gel for 20 min at a voltage of 5 V/cm. The results were subsequently analyzed. Specific primers were utilized to amplify different regions of the 16S rRNA gene. The thermal cycling steps during amplification involved an initial denaturation at 98°C for 1 min, followed by denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s. The final extension step was held at 72°C for 5 min. All PCR reactions were performed using a 15-µL Phusion high-fidelity hybrid PCR instrument (New England Biolabs) with 0.2 µM of both forward and reverse primers, and approximately 10 ng of template DNA. The PCR products were mixed with an equal volume of IX carrier solution containing SYB green and subsequently detected by electrophoresis on a 2% agarose gel. The PCR products were then mixed in equal density proportions and purified using the Qiagen Gel Extraction Kit (Qiagen, Germany).

The obtained 16S rRNA gene sequences were checked against the Mothur software (version 1.35.1, http://www.mothur.org/wiki/Download_mothur) database, and optimized sequences were obtained after thorough removal of chimeras. These optimized sequences were further classified into Operational Taxonomic Units (OTUs) with 97% similarity. Dilution curve analysis was conducted based on the OTUs, and the richness index (Chao1) and diversity index (Shannon) were calculated. The representative sequences of OTUs with 97% similarity were taxonomically analyzed using the Bayesian algorithm of RDP classifiers against the Silva Bacterial Database, and the samples were counted at each taxonomic level, including kingdom, phylum, class, order, family, genus, and species. The community composition was analyzed using Pearson's correlation analysis to investigate potential associations between different rinsing and disinfection methods and the microbial community structure of vehicles. Finally, high-throughput sequencing was performed on the Illumina NovaSeq platform.

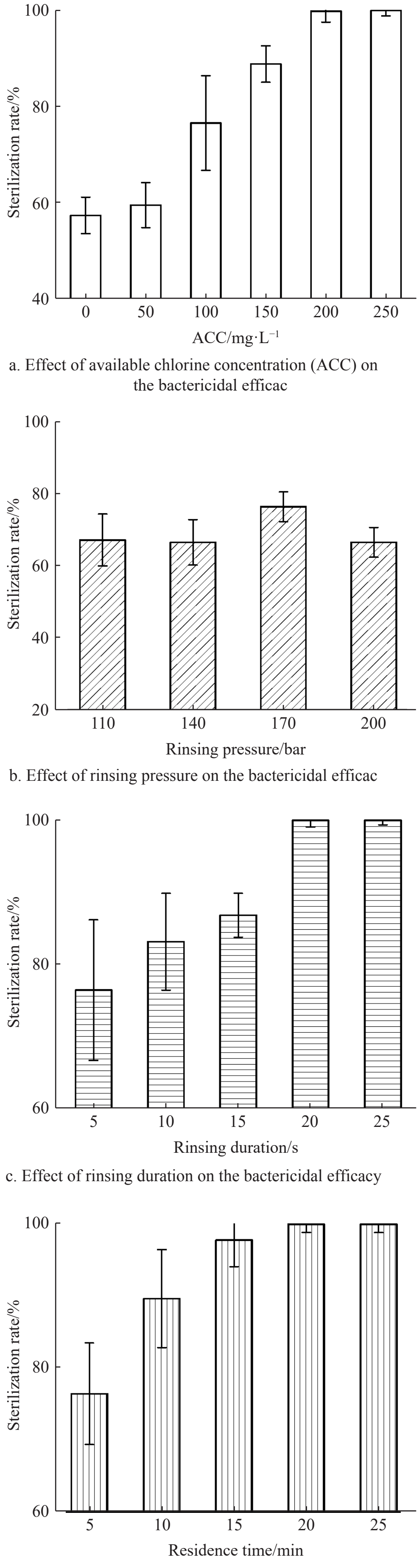
3 Results and discussion

3.1 Single factor test of parameters on bactericidal rate

To examine the effect of the operating method of SAEW on sterilization efficacy under high pressure, a single factor test was conducted to investigate each parameter. Firstly, pressure, rinsing duration, and residence time were set to 170 bar, 5 s, and 5 min, respectively. Figure 2a illustrates the bactericidal rate following the working of SA-HP with tap water (ACC of 0.6 mg/L, represented by 0 mg/L) and SAEW at varying ACC of 50, 100, 150, 200, and 250 mg/L. The bactericidal rate of tap water was (57.32±3.79)%, and that of SAEW with an ACC of 50 mg/L was (59.47±4.67)%. The difference between them was not significant (p>0.05). When the ACC reached 100 mg/L and above, there was a significantly different in the sterilization efficacy compared to tap water (p<0.05). The bactericidal effect of SAEW on the surface of tires increased as the ACC rose, and the bactericidal rate reached 100% at 200 mg/L ACC. The optimal ACC range of SAEW for high-pressure rinsing should be maintained between 100 and 200 mg/L. HPWJ had effect on the removal of pathogens and pollutants from the surface of the facility by itself. In addition, the high pressure condition does not diminish the sterilization efficiency of the SAEW, so the characteristic that the sterilization effect varies with ACC is still maintained.

Secondly, Figure 2b depicts the sterilization rate at pressures of 110, 140, 170, and 200 bar, with an ACC of 100 mg/L, a rinsing duration of 5 s, and a residence time of 5 min. There was no significant difference in the sterilization effect under different pressures (p>0.05). The cleaning property of HPWJ does not depend on the rinsing pressure alone, but rather on consideration of the rinsing pressure and the target distance, incidence angle, rinsing time, and other non-structural parameters of the interaction between the rinsing pressure and target based on the principle of HPWJ. Not only does excessive rinsing pressure result in significant energy loss, but it also increases the operational risk. Therefore, in order to ensure the effectiveness of sterilization as well as to reduce the energy consumption, it is recommended that the rinsing pressure used in the SA-HP process be approximately 170 bar.

Thirdly, Figure 2c shows the variation in bactericidal rate of different rinsing duration with ACC, pressure, and residence time of 100 mg/L, 170 bar, and 5 min, respectively. The sterilization effect of SA-HP on the tire surface increased with the extension of rinsing time, with a clear trend of increasing bactericidal rate between 5 and 15 seconds. After the rinsing time reached 15 seconds and above, the bactericidal effect tended to stabilize until the sterilization effect was no longer significant (p>0.05). This is consistent with the findings of other studies on the law of surface disinfection[21]. The range of rinsing duration applicable to SA-HP process should be between 10 and 20 seconds in order to achieve the highest sterilization effect in the shortest possible period of time. After recalculation, the rinsing speed is 0.0064-0.0128 m^2/s and the rinsing duration required per square meter of surface area is 78-160 s.



a. Effect of available chlorine concentration (ACC) on the bactericidal efficac

b. Effect of rinsing pressure on the bactericidal efficac

c. Effect of rinsing duration on the bactericidal efficacy

d. Effect of residence time on the bactericidal efficacy

Figure 2 Effects of operating parameters on the bactericidal efficacy of SAEW

Lastly, the effect of different residence time on the sterilization rate at ACC of 100 mg/L, pressure of 170 bar and rinse duration of 5 s is shown in Figure 2d. Between 5 and 15 min, the sterilization rate grew substantially. After the residence time reached 15 min or more, there was no significant difference under residence time (p>0.05). The post-rinsing residence time for SA-HP should be between 10 and 20 min in order to obtain the greatest sterilization effect with the shortest operation time. Several studies have demonstrated that the cleaning effect of a HPWJ was constrained by structural parameters such as the high-pressure equipment and the size and shape of the nozzles[22]. However, non-structural parameters such as the rinsing pressure and time can also affect the cleaning efficiency. A reasonable choice of non-structural parameters can effectively improve the energy utilization and the equipment's structural performance. Therefore, various working parameters have a practical influence on the sterilization rate of SAEW under high pressure working conditions, revealing that effective parameter settings can boost the sterilization effect of innovative SA-HP application technology. Research has become preoccupied with determining how to combine the operation parameters.

3.2 Orthogonal test of sterilization effect of combined process

Orthogonal tests were designed in the animal environment simulation laboratory based on Table 1. The sterilization effect was evaluated by measuring the total plate count present on the tire surface before and after conducting the experiment. The test results are listed in Table 2, indicating that the highest sterilization rate was achieved in test 7, reaching (98.47±0.64)%, and the lowest was observed in test 1, with a value of (82.72±1.19)%. It is noteworthy that, apart from test 1 and test 5, all other combinations of process parameters can reach more than 90% of the sterilization rate. It was observed that the third level of ACC (A) at 200 mg/L was superior to the other two levels (150 mg/L, 175 mg/L). Similarly, the third level of rinsing duration (B) at 20s exhibited higher efficacy compared to the other two levels (10 s, 15 s). Furthermore, the second level of residence time (C) at 15 min demonstrated better results than the other two levels (10 min, 20 min). Based on the range R, it can be inferred that the factors influencing the sterilization rate, in order of priority, are ACB. Among these, the degree of influence is ACC>residence time>rinsing duration. According to the results of the orthogonal test, the bactericidal rate of the test group with a rinsing time of 10 s has already reached (98.47±0.64)%, and a shorter rinsing time can effectively reduce the cost of rinsing and sterilization. Hence, it is advisable to employ a combination of an ACC of 200 mg/L, a rinsing duration of 10 s, and a residence time of 15 min for the purpose of cleaning and sterilization in practical applications.

Based on the principle of HPWJ, when the distance between the nozzle and the target is shorter than the optimal target distance, the object is within the range corresponding to the initial stage of the jet process[23]. The object experiences a substantial impact force, while the surface area affected by the jet is relatively limited. In the scenario where the flushing pressure remains constant, the scope of the object is influenced by the force exerted by the jet as the distance between the jet and the target increases. This force increases until the optimal distance between the nozzle and the target is reached. At this point, the force exerted by the jet on the object reaches its maximum value[24]. Nevertheless, if the distance between the nozzle and the target surface surpasses the ideal target distance, the jet transitions into the diffusion phase. During this stage, the jet experiences heightened air turbulence, causing the fluid at the boundary of the jet to disintegrate into a mist of droplets.

Consequently, the impact force of the jet on the object diminishes as the target distance increases within this phase. Based on the findings of the conducted simulation tests, it is recommended that a pressure of 170 bar is used for the purpose of conducting flushing operations in practical production scenarios.

Table 2 Analysis of orthogonal test

Test number	Factor A ACC/mg·L^{-1}	Factor BRinsing duration/s	Factor C Residence time/min	Sterilization rate/%
1	150	10	10	82.72±1.19
2	150	15	15	91.65±1.08
3	150	20	20	92.18±0.83
4	175	10	20	96.21±0.97
5	175	15	10	89.68±1.52
6	175	20	15	97.00±1.01
7	200	10	15	98.47±0.64
8	200	15	20	98.41±0.59
9	200	20	10	96.77±0.92
S1	266.55	277.40	269.17	-
S2	282.89	279.75	287.13	-
S3	293.65	285.95	286.8	-
K1	88.85	92.47	89.72	-
K2	94.30	93.25	95.71	-
K3	97.88	95.32	95.60	-
R	9.03	2.85	5.99	-
Optimal combination		A3B3C2		

Since the surface of the tire model can easily conceal dirt, the livestock and poultry facilities have more areas that cannot achieve effective cleanliness. High pressure can fully release hypochlorite's oxidizing activity, and atomized SAEW may effectively boost the bactericidal capacity to ensure the biosafety of the farm by making thorough contact with the microorganisms on the facilities and various production processes.

3.3 Cleaning and sterilizing effect of SA-HP

A field test was conducted at the pig transfer vehicle decontamination center of a pig farm to examine the sterilization efficacy of the traditional method and the SA-HP system. The initial colony count on the surface of the vehicle, without any rinsing or sterilization treatment, was found to be (4.56±0.28) lg CFU·cm^{-2}. Following the application of the traditional method, the colony count decreased to (3.73±0.22) lg CFU·cm^{-2}. Furthermore, after the implementation of the SA-HP treatment, the colony count decreased to (2.10±0.19) lg CFU·cm^{-2}. The sterilization rates were (85.30±2.85)% and (96.50±0.91)%, respectively, and both methods had a significant influence on reducing the number of colonies present on the surface of the vehicle ($p<0.05$). The SA-HP system showed a higher level of effectiveness in comparison to the traditional method. Research has demonstrated that the bactericidal rate of disinfectants is influenced by the size of droplet particles[25]. The application of high pressure conditions in SAEW brought about several advantages, including the generation of smaller droplet sizes, increased frequency of contact, and subsequently enhanced efficiency of dealing with the complex cleaning requirements associated with pig transfer vehicles. Residual microorganism can occur from the extended application of traditional methods on the vehicles present at the site, leading to the development of a specific level of drug resistance among the flora organisms[26]. Simultaneously, it should be noted that the foam detergent employed in the traditional method possesses alkaline properties, which can potentially result in the presence of alkaline residue on the vehicle surfaces and diminish the bactericidal efficacy of the acidic disinfectant. Traditional methods utilize a diverse array of detergents and disinfectants, which have the potential to interact in an antagonistic reaction. And water consumption and workload associated with traditional methods are very high, thereby impeding energy conservation and environmental preservation efforts. Take a pig transfer vehicle with a length of 6.8 m, a width of 2.4 m, and a height of 2.6 m as an example. In traditional methods, a series of procedures included cleaning, drying, and disinfection, and it repeated at least twice. The duration of the cleaning operation spans approximately 2 h. On average, each vehicle generates a volume of sewage containing nitrogen and phosphorus components ranging from 0.7 to 1 t. The SA-HP technology exhibits a sewage generation rate per vehicle of approximately 0.3 t, while the SAEW will be reduced to regular water. In terms of sterilization efficacy and cleaning efficiency, the SA-HP of rinsing and disinfection has the potential to fully substitute the traditional method. Consequently, the application of SA-HP would enhance operational efficiency, minimize the utilization of chemicals, and reduce the demand for sewage treatment.

3.4 The effect of this technique on the community structure

The samples collected from test vehicles were analyzed by 16S rRNA high-throughput sequencing before and after cleaning. The normalized sequences were classified according to a similarity level of 97%, resulting in a total of 4012 OTUs. 2355, 861, and 796 OTUs were obtained in the control, traditional, and SA-HP groups, respectively. The OTUs were reduced by more than half in samples treated with two different rinsing procedures, which indicated that the abundance of the bacterial community on the surface of the vehicles was reduced as a result of the cleaning treatment. The SA-HP group exhibited the lowest OTUs, which suggests that the SA-HP method had a notable impact on reducing the abundance of bacterial communities on the surface of the vehicle. The α-diversity indices, including Shannon and Chao1 indices were also analyzed in each group of samples. The Shannon indices for the control, traditional, and SA-HP groups were determined to be 7.56, 6.73, and 6.40, respectively. Similarly, the Chao1 indices for the control, traditional, and SA-HP groups were 862.58, 801.39, and 787.89, respectively. The findings from the SA-HP group demonstrated that this innovative sterilization method effectively decreased the abundance and diversity of bacterial flora from the vehicles, as indicated by the smallest Shannon and Chao1 indices observed. Compared to the traditional method, as evidenced by the OTU results, SA-HP technique provides better control of microorganisms on facility surfaces, thereby lowering the potential risks associated with infection and transmission.

The community structure of the control, traditional, and SA-HP groups were analyzed for relative abundance. At the phylum level, the detected species were ranked in terms of their relative abundance as follows: *Firmicutes*, *Bacteroidota*, *Proteobacteria*, *Actinobacteriota*, *Euryarchaeota*, *Desulfobacteria*, *Campilobacterota*, and *Cyanobacteria*. These were typical intestinal flora, indicate that the pathogenic microorganisms present on the transport vehicle's surface primarily originated from animal feces. This result was mostly consistent with the microorganisms of samples collected on vehicle tires. Figure 3 showed the changes in the community structure on the vehicle surface before and after traditional method and SA-HP treatment at the genus level. In the samples before the treatment, a total of 13 microbial species were identified with a relative abundance exceeding 1%. Among these species, *Ignavigranum* exhibited the highest relative abundance of approximately 14.58%, which was found to be the predominant genus present on the surface of the vehicles. Following *Ignavigranum*, the genera *Anaerococcus*, *Helcococcus*, *Clostridia*, *Facklamia*, and *Aerococcus* were also observed in notable proportions. After the traditional method, there was a significant decrease in the relative abundance of *Ignavigranum* to 2.42%. Additionally, the predominant genus in the samples changed to *Clostridia*, which exhibited a relative abundance value of 19.68%. After the SA-HP treatment, there was a notable reduction in the relative abundance of *Ignavigranum*, which reached a value of 3.03%, and the predominant genus observed in the samples shifted to *Clostridia* with a relative abundance of 11.26%. The antibacterial effect of SA-HP system against *Ignavigranum*, *Anaerococcus*, *Helcococcus*, and other pathogenic bacteria is noteworthy, aligning with previous reports that SAEW exhibits bactericidal properties against pathogenic bacteria in poultry facilities[27]. The HPWJ method effectively eliminates organic matter and enhances the performance associated with SAEW. The high-pressure condition promotes greater interaction between hypochlorite and pathogenic bacteria, thereby improving the bactericidal efficacy and maintaining it over an extended duration. The effective implementation of SA-HP technology facilitates advancements in sterilization techniques within agricultural settings.

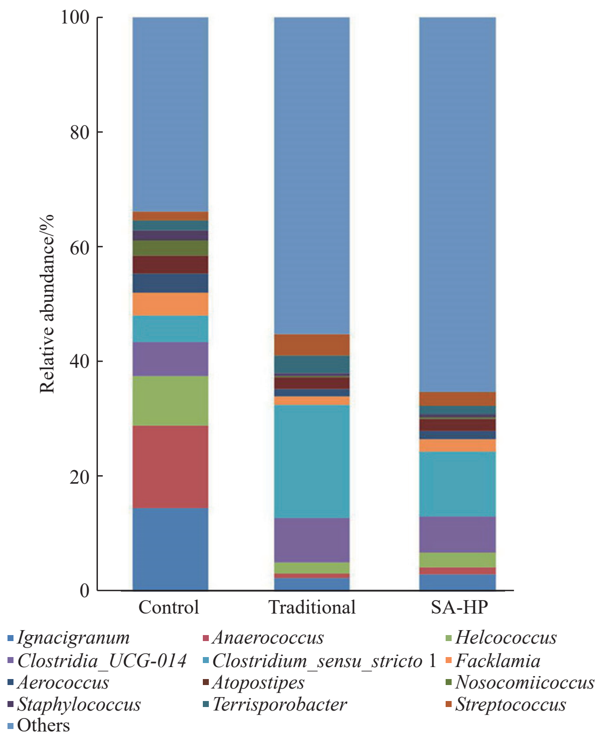


Figure 3 Community composition variations from different cleaning methods at the genus level

4 Conclusions

This study presents a novel rinsing and sterilization technology SA-HP, analyzed the influencing factors and the regulation of operation parameters on the sterilizing effect. Furthermore, the study evaluates the practical application of this technology in sterilizing transfer vehicles in pig farms. The sterilization efficacy of SAEW was significantly improved under high-pressure conditions, as influenced by three non-structural parameters: ACC, rinsing duration, and residence time. The relationships between these influencing factors can be sorted as follows: ACC>residence time>rinsing duration. After the optimization of the process, the suggested parameter combinations consisted of a rinsing pressure of 170 bar, an ACC of 200 mg/L, a rinsing duration of 10 s, and a residence time of 15 min. And the application of the SA-HP technique enabled an apparent reduction in the surface colony concentration of pig transfer vehicles, with an average value of (2.10±0.19) lg CFU·cm^{-2}, and achieved a bactericidal rate of (96.50±0.91)%. The alternative approach is more efficient in terms of time and effort, and it generates less sewage compared to the traditional method. SA-HP technology has a major effect on reducing the bacterial diversity present on the surfaces of transfer vehicles and can effectively inhibit the transmission of pathogenic bacteria such as *Ignavigranum*, *Anaerococcus*, *Helcococcus*, etc. The development of SA-HP process is of high value in the sterilization study in complex environments. The application domains of SAEW and HPWJ technologies are expanded, and technical obstacles are effectively overcome. This technology demonstrates significant potential in preventing the spread of diseases across different stages of production in livestock and poultry farming operations. Consequently, it offers technical support to ensure the biosafety and facility sanitation of concentrated animal production in an energy-efficient, sustainable and cost-reducing manner.

Acknowledgements

The authors are grateful for the financial support of this project by the Strategic Priority Research Program of the National Center of Technology Innovation for Pigs (Grant No. NCTIP-XD/B07).

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