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Article

Performance of a Combined Treatment Approach on Elimination of Microbes from Poultry Slaughterhouse Wastewater

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Abstract: The efficiency of microbial inactivation in water is highly dependent on the type of treatment technology used as well as the characteristics of the water to be treated. Wastewater from poultry slaughterhouses carries a significant number of microorganisms posing threat to humans and the environment in general. Therefore, the treatment of poultry slaughterhouse wastewater requires the use of appropriate purification systems with high removal efficiency for microbial agents. In this study, the performance of an integrated treatment plant with electrolysis, ultrafiltration, and ultraviolet radiation as the principal treatment units is investigated in terms of microbial inactivation from poultry slaughterhouse wastewater. In this case, Total microbial number, Total coliform bacteria, Thermo-tolerant coliform bacteria, Pathogenic flora, including Salmonella coliphages, Spores of sulfite-reducing clostridia, Pseudomonas aeruginosa, and Staphylococcus aureus Enterococcus were studied. About 63.95% to 99.83% of the microbes were removed by the EC treatment unit, as well as 99.86% to 100% removal efficiency was achieved after the combined treatment. However, the Pseudomonas aeruginosa was the only microbial agent detected in the final effluent after the combined treatment. The phenomenon suggests that an upgrade to the treatment plant may be required to achieve 100% removal assurance for Pseudomonas aeruginosa.

Keywords: Electrochemical; integrated wastewater treatment; microorganisms; poultry slaughterhouse; ultrafiltration; ultraviolet radiation

1. Introduction

The process of slaughtering chickens is associated with the consumption of large quantities of water from the meat processing activities, cleaning of the processing environment, disinfection as well as transportation of the slaughter by-products. The processes also generate large quantities of highly polluted wastewater with organic matter including pathogenic microorganisms [1]. As the population increases around the world, the demand for poultry products has also been increasing which in turn affects the general water demand as well as increasing the generation of highly polluted wastewater [2]. The increasing cost of discharging untreated process water into the local sewage systems, as well as the presence of more restrict requirements for discharging process water onto surface water [3], has made many poultry farms in the world to think of the in-situ treatment of the wastewater generated from slaughterhouses. Microbes are among the contaminants of significant concern generated from the poultry slaughterhouses [4].

Biological contaminants are of different types including different types of bacteria such as *Fecal coliforms* and *Escherichia coli* [5], *Salmonella*, *Shigella*, *Vibrio cholerae*, as well as *Pseudomonas aeruginosa* [6]. Also, some other biological contaminants such as viruses, fungi as well as diverse parasite cysts and eggs can be found in the wastewater. The degree to which the biological contaminants may pose threat to environmental and human health is dependent on the type and concentration [7].

There are many health risks associated with exposure to biological contaminants in water including diseases such as typhoid, cholera, and tuberculosis caused by bacteria [8], hepatitis caused by viruses [9], as well as dysentery caused by protozoa [10]. Therefore, it is of great importance to ensure that the wastewater from poultry slaughterhouses has been adequately treated to achieve as complete as possible elimination of biological contaminants before either discharge or any other utilization. In general, there are many technologies used for microbial elimination [11–13]; conventional technologies are the most widely used processes for disinfection of water. Chlorine, chlorine dioxide, ozone, and peracetic acid are a few examples of the chemical-based microbial elimination approaches. In addition to chemical disinfectants, ultraviolet (UV) radiation has also been used for many years as a water disinfection technology in the field of wastewater treatment [14]. Also, some other advanced approaches such as ozonation and membrane filtration [15] as well as electrochemical (EC) methods [16] have been applied to poultry slaughterhouse wastewater treatment. But, the performance of the treatment technologies has also been observed to be affected by the scale and characteristics of wastewater subjected to the treatment process [17]. With the fact that each treatment system is characterized by its advantages and disadvantages in terms of strengths and weaknesses, it is preferable to combine several treatment technologies to form an integrated treatment to achieve higher treatment efficiency for poultry slaughterhouse wastewater [18]. Previous studies have observed that a combination of different technologies has the potential to improve the general performance of a treatment system as pollutants that were not removed by one unit can be removed by the other subsequent units [19]. EC, ultrafiltration (UF), and UV are among the treatment technologies used in poultry slaughterhouse wastewater treatment. However, the information about their technical feasibility on microbial elimination from poultry slaughterhouse wastewater is still scarce especially when integrated together.

EC treatment systems have been considered as cost-effective and highly efficient wastewater treatment technologies. The EC treatment systems have been observed to be highly efficient in the removal of pollutants from poultry slaughterhouse wastewater [20,21]. Despite the EC treatment technologies being extensively studied for recalcitrant organics removal, its application potential towards microbial elimination from wastewater such as the one generated from poultry slaughterhouse processes is still not well known [22].

Generally, UF is a pressure-driven membrane separation mechanism that removes suspended particulate matter and some dissolved compounds with high molecular weight, including organics and colloids from wastewater [23]. UF is also regarded to be effective in removing bacteria and most viruses present in wastewater [24]. The efficiency of UF systems on filtering out microorganisms from wastewater makes the technology ideal for the treatment of poultry slaughterhouse wastewater. Moreover, the UF treatment systems are also useful when applied as pre-treatment units before reverse osmosis, UV, and ozone treatment systems as disinfection requirements are greatly reduced due to the reduction in suspended solids [25].

Unlike the chemical approaches to water disinfection, UV light has been observed to offer a more rapid and effective inactivation of microorganisms through a physical process [26,27]. The UV rays must strike the cell to inactivate microorganisms in water. UV

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energy breaches the outer cell membrane of the microorganism, of which in the process the DNA is disturbed which in turn prevents reproduction [28]. Among the crucial benefits of water treatment with UV is the fact that the treatment process does not alter water chemically as no additional chemicals are added except energy [29]. However, unlike the membrane filtration treatment systems, the sterilized microorganisms with UV rays are not removed from the water. It should also be noted that generally, the UV disinfection process does not remove dissolved organics, inorganics, or any other sort of particles in the water. When the water is subjected to UV rays, the microorganisms exposed to the germicidal wavelengths of UV light, are rendered incapable of reproducing and infecting making them harmless [30].

In this study, an integrated lab-scale treatment plant with EC, UF, and UV is studied for its potential application towards microbial elimination from poultry slaughterhouse wastewater. The wastewater samples collected from the Izevski Production Corporative (PC) poultry farm slaughterhouse in Kazakhstan were treated using the treatment plant installed in the Water and Environmental Management laboratory at the L.N. Gumilyov Eurasian National University in Nur-Sultan, Kazakhstan.

2. Materials and Methods

2.1 Case study, sample collection, and wastewater characteristics

The wastewater samples used in this study were collected from the Izhevsk PC poultry slaughterhouse located in Izhevsk village, Arshalinsky district, in Akmola region of the Republic of Kazakhstan, about 70km from the capital city Nur-Sultan (51° 10' North latitude and 71° 26' East longitude). Samples were collected as grab samples using 5L plastic bottles, which were thoroughly rinsed with deionized water before use. All samples were preserved at 4 °C for transportation and before treatment in the laboratory. Table 1 highlights the general characteristics of the wastewater in terms of microbial contaminants. A total of nine (9) microbial parameters (*Total microbial number (TMN), Total coliform bacteria (TCB), Thermo-tolerant coliform bacteria (TTCB), Pathogenic flora, including salmonella coliphages, Spores of sulfite-reducing clostridia, Pseudomonas aeruginosa, and Staphylococcus aureus Enterococcus*) were studied.

Table 1. Wastewater characteristics

Microorganisms	Min	Max	AM	Median	SD
TMN	1254	2305	1780	1780	429.07
TCB	1122	2450	1991	2400	614.58
TTCB	659	1020	793	700	161.38
Pathogenic flora, including *salmonella*	55	93	78	85	16.36
Coli phages	30	48	37	32	8.06
Spores of sulfite-reducing clostridia	53	90	71	70	15.12
Pseudomonas aeruginosa	2500	4020	3197	3070	626.97
Staphylococcus aureus	2030	3987	2935.67	2790	805.55
Enterococcus	1022	2090	1396.67	1078	490.79

TMN in CFU/ml, all other parameters in CFU/100ml; Min= minimum, Max= maximum, AM= average mean, SD= standard deviation

2.2 Experimental setup

The treatment plant is composed of EC, UF, and UV as the main treatment units installed in series. Each experimental session used 1.7 L of wastewater. The experiments started by treating the wastewater samples from defeathering and cooling sections of the slaughterhouse separately followed by the experiments from the mixed wastewater. The main treatment process starts from the EC unit by applying a unipolar voltage to the metal plates-electrodes from the power unit. The effluent from the EC unit is then subjected to the UF treatment process, preparing the wastewater for UV disinfection.

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2.2.1 EC treatment

The EC treatment process was done in a container of 15 × 13 × 11 cm^3 dimension made of polypropylene material in which the electrodes are placed. Direct current in a potentiostatic mode was applied to both electrodes in the EC container, with a design power supply ranging from 0 to 50 V for voltage and 0 to 10 A for current density (Xinhua Electrical Weld Company, Loudi City, China). In this study, aluminum was used as an anode electrode with dimensions of 10.8 × 11.8 × 0.2 cm^3, while titanium was used as a cathode electrode with dimensions of 10.8 × 11.8 × 0.7 cm^3. To avoid variability, the distance between electrodes was fixed to 2cm in all sessions of the experiments and were placed parallel to the reactor. The other general technical specifications are summarized in Table 2.

Table 2. EC technical specifications.

Parameter	Value	Unit
Initial water temperature	5–10	°C
Potential (voltage)	24	V
Average current density	5.5	A
Average power	132	W
Hydraulic retention time	40	min

2.2.2 Ultrafiltration

The treatment process is followed by the mechanical UF which plays an important role in preparing the pre-treated wastewater for UV disinfection. In this process, some of the particles that escaped from the previous treatment units are removed. The UF treatment process is achieved by passing the effluent from the EC unit through a cartridge-type filter for an ultrafine cleaning. The transboundary pressure for the water to flow through the filter material with a pore size of 0.02 µm or 1760 kDa (Aquafor LLP, Moscow, Russia) is generated by the NS1 pump (Pionerskaya str 27A, Saint Petersburg, Russia) installed within the UF compartment. Table 3 provides a general summary of the technical specifications from the UF treatment unit.

Table 3. UF technical specifications

Parameter	Unit	Value
Filter pore size	µm	0.02
Pump supply voltage	V	24
Pump power	kW	0.2-0.4

2.2.2 UV disinfection

After the treatment processes from EC and UF units, the wastewater is subjected to the UV disinfection unit, to eliminate the remaining microorganisms. The basic principle of this UV sterilization is that water is exposed to UV radiation to inactivate microbes in the water. The UV sterilizer is installed within a cylindrical container made of stainless steel equipped with input and output nozzles. Inside the container sealed with a plastic knob, there is a quartz tube mounted, with a quartz germicidal lamp is installed inside. The general purpose of the quartz tube is to avoid direct water contact with the lamp and easy replacement without the need to drain liquid from the UV container. The UV sterilization unit has been properly designed to sustain water pressure during the purification process. Valves have also been installed to control and adjust the flow of water during the treatment process.

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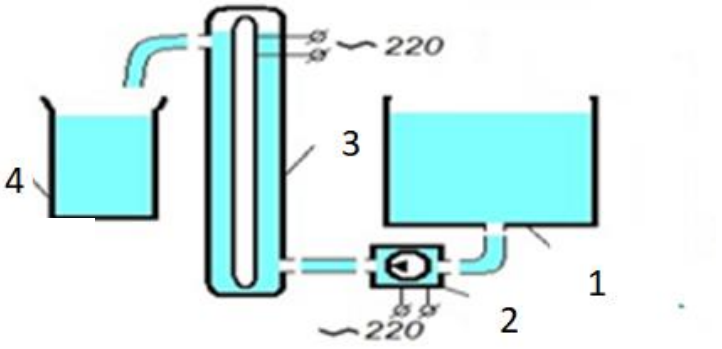

Figure 1. Photochemical Treatment System: 1- Wastewater reservoir, 2- Small pump, 3- UV sterilizer, 4- Purified water reservoir.

Figure 2. Lab-installed UV disinfection unit

2.3 Analytical methods

The microbial analysis was achieved using the membrane filtration method [31], where the water samples before and after the treatment processes were passed through a membrane filter with a pore size of 0.45 µm and then incubated on an agar plate at 37℃ for 48 hours. In general, the microbial analysis of the water samples was accomplished following the recommendations in the APHA, Standard Methods for the Examination of Water and Wastewater [32].

2.4 Statistical methods

The results from the microbial analysis were subjected to statistical analysis for an easy interpretation of the results. The statistical analysis included computation of removal efficiencies for each of the studied microbial parameters. The removal efficiencies were computed in terms of percentages, to investigate the performance of the treatment approach with respect to the studied microbial parameters. The approach used for the treatment efficiency analysis is summarized in (1).

$$T_e(\%) = \frac{C_b - C_a}{C_b} \times 100\% \quad (1)$$

3. Results and discussion

The wastewater samples treated using the combination of EC, UF, and UV were successfully analyzed. Tables 4 and 5 provide a summary of the microbial analysis results presented in terms of minimum values (Min), maximum (Max), arithmetic mean (AM), median as well as standard deviation (SD).

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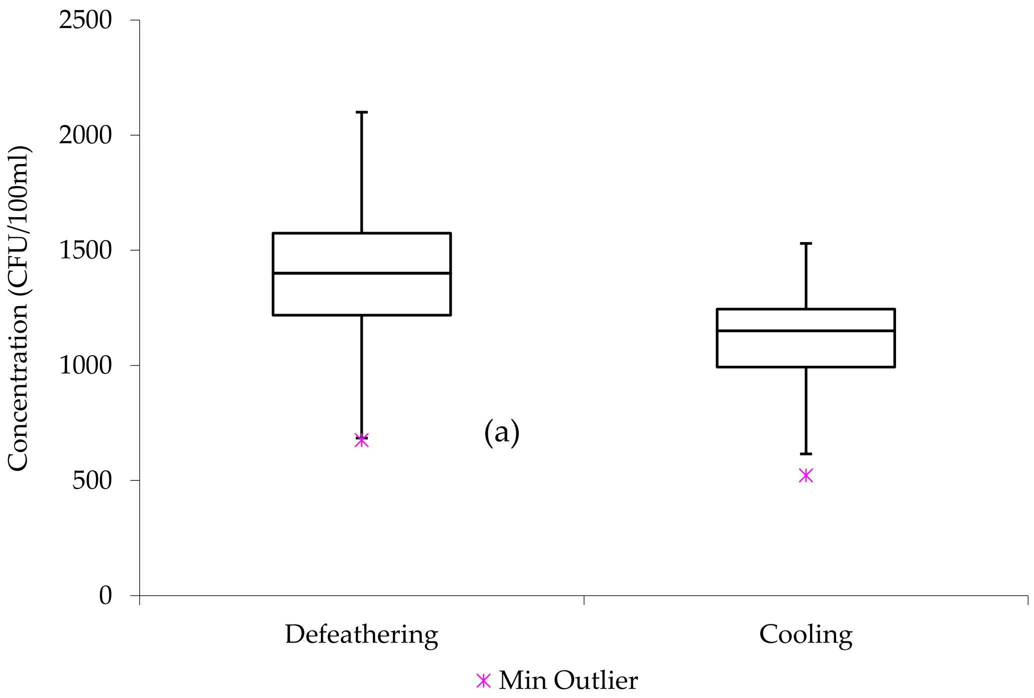
Figure 3 shows the trend of microbial concentrations in the raw wastewater for the case of TMN and TCB from the defeathering and cooling wastewater samples. From Figure 3(a), it can be observed that the TMN concentration in the defeathering wastewater is more of symmetric distribution with the median observed to be closer to the middle. The boxplot from the cooling section wastewater samples shows the median closer to the upper or top quartile; in that matter, the distribution of microbial concentration in the studied wastewater samples is considered to be "negatively skewed".

From Figure 3 (b) defeathering boxplot, the median is observed to be closer to the lower quartile with an indication that the TCB concentration data from the studied wastewater samples constitute a higher frequency of more high concentration values than the low concentration values ("positively skewed"). While that of the cooling section observed to be symmetric.

In general, Figure 3 reveals further that the wastewater from the defeathering section is more contaminated with microbes than the wastewater from the cooling section of the poultry slaughterhouse. The phenomenon can be highly linked to the nature of production activities between the two sections, of which the defeathering process is regarded to be generating wastewater with a higher organic load than the cooling wastewater [33].



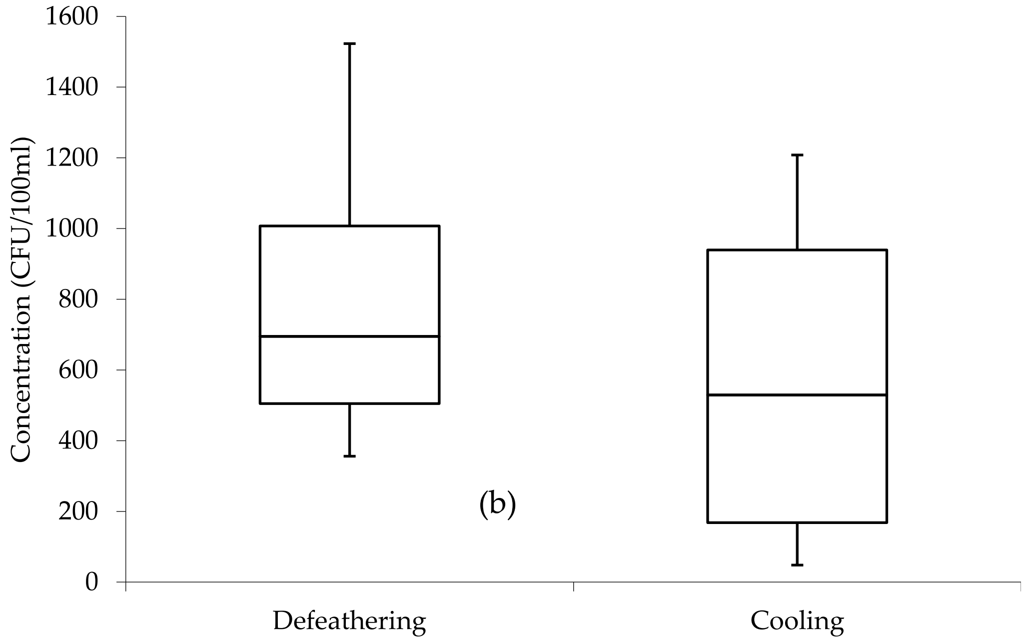


Figure 3. Microbial concentrations from defeathering and cooling sources (a) TMN in raw wastewater (b) TCB in raw wastewater

TMN and TCB were the main microbial parameters investigated when the wastewater samples from defeathering and cooling sections of the slaughterhouse were treated separately. From Figure 4, it can be observed that generally the integrated treatment plant achieved more than 99.73% of microbial removal efficiency from both defeathering and cooling wastewater samples. The deviation in terms of removal efficiency for TMN and TCB from both defeathering and cooling sources is literary small. The results indicate that the treatment approach can be highly effective even when subjected to wastewater with fluctuating pollution load.

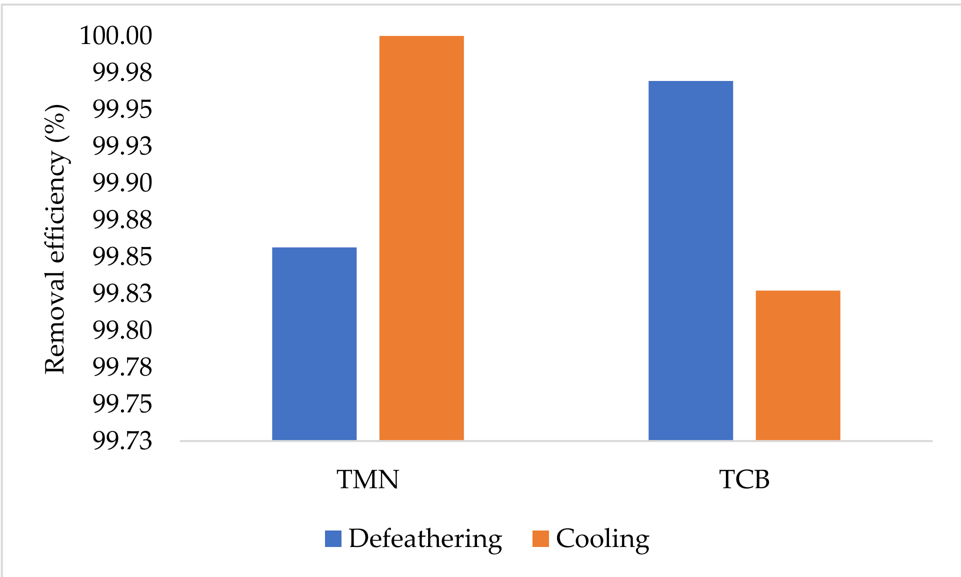


Figure 4. TMN and TCB removal efficiencies from defeathering and cooling sections of the poultry slaughterhouse

3.1 EC effluent quality

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Samples were collected immediately after the EC treatment and analyzed to investigate the performance of the EC treatment unit in terms of microbial inactivation. From Table 4, it can be observed that in some cases the EC treatment unit was able to eliminate all the microbes in wastewater with zero (0) microbial count being achieved as the minimum concentration value for TCB, TTCB, *Pathogenic flora*, including *Salmonella*, *Coli phages*, *Staphylococcus aureus*, as well as *Enterococcus*. In general, the lowest average concentration value from the EC effluent is observed from the *Coli phages* achieving 1 CFU/1000ml. However, it should also be noted that the concentration of *Coli phages* was observed to be generally low in raw wastewater. Moreover, the EC treatment unit faced a significant challenge in the removal of *Pathogenic flora*, including *salmonella*. The average concentration of *Pathogenic flora*, including *salmonella* was 78 CFU/100ml in the raw wastewater, while the EC treatment unit was able to reduce the concentration to 28 CFU/100ml., which can be termed as low performance in comparison to the other studied microbial parameters.

Table 4. Microbial results from EC effluent

Microbial parameter	Min	Max	AM	Median	SD
TMN	34	100	62	50	28.11
TCB	0	36	20	23	14.88
TTCB	0	87	29	0	41.01
Pathogenic flora, including *salmonella*	0	84	28	0	39.60
Coli phages	0	2	1	0	0.94
Spores of sulfite-reducing clostridia	2	5	4	4	1.25
Pseudomonas aeruginosa	20	46	31	25	11.26
Staphylococcus aureus	0	16	6	1	7.32
Enterococcus	0	4	3	3	1.70

TMN in CFU/ml, all other parameters in CFU/100ml

3.2 Final effluent quality

From Table 5, it can be observed that after the combined treatment, the treatment plant was able to eliminate all the microbes in the wastewater for all the studied microbial parameters with expect to *Pseudomonas aeruginosa.* For the case of *Pseudomonas aeruginosa*, the treatment plant did not achieve 0 CFU/100ml during some experimental sessions and the microbial parameter was generally observed to be the most resistant group among the studied microbial parameters. On average, a microbial count of 5 CFU/100ml for *Pseudomonas aeruginosa* was recorded in the final effluent after the combined treatment. The maximum recorded *Pseudomonas aeruginosa* microbial count was 13 CFU/100ml. However, in some experiments, the treatment approach achieved 0 CFU/100ml for *Pseudomonas aeruginosa* as observed in Table 5.

In general, despite the challenge with *Pseudomonas aeruginosa*, the treatment approach was able to achieve 0 CFU as the minimum recorded concentration value for all the studied microbial parameters.

Table 5. Microbial results from the final effluent

Microbial parameter	Min	Max	AM	Median	SD
TMN	0	0	0	0	0
TCB	0	0	0		0
TTCB	0	0	0	0	0
Pathogenic flora, including *salmonella*	0	0	0	0	0
Coli phages	0	0	0	0	0
Spores of sulfite-reducing clostridia	0	0	0	0	0
Pseudomonas aeruginosa	0	13	5	0	6.13

Staphylococcus aureus	0	0	0	0	0
Enterococcus	0	0	0	0	0

TMN in CFU/ml, all other parameters in CFU/100ml

3.3 General removal efficiency

From Figure 5 it can be observed that, in terms of the removal efficiency, on average, the EC treatment unit was able to remove the majority of the microorganisms with efficiency ranging from 64.1% to 99.83%. In the raw wastewater, an average of 1780 CFU/ml of TMN was recorded, after the EC treatment an average of 62CFU/ml was recorded which is equivalent to 96.52% removal efficiency. For the TCB, an average of 1991CFU/100ml was recorded in the raw wastewater, with 20 CFU/100ml recorded as an average count after the EC treatment which is equivalent to 99% removal efficiency. Moreover, an average of 793 CFU/100ml of TTCB was recorded in the raw wastewater, while after the EC treatment an average count of 29 CFU/100ml was recorded, equivalent to 96.34% removal efficiency. An average of 78 CFU/100ml of *Pathogenic flora,* including *salmonella* was recorded from the raw wastewater, while an average of 28 CFU/100ml of *Pathogenic flora,* including *salmonella* was recorded after the EC treatment, equivalent to 64.1% removal efficiency.

Also, from the combined treatment the treatment plant achieved almost 100% removal efficiency for all the studied microbial parameters, except for the *Pseudomonas aeruginosa* in which an average of 99.84% removal efficiency was achieved. According to the literature [34–36], the high removal resistance observed from the *Pseudomonas aeruginosa* can be highly linked to the ability of the bacteria to form a biofilm, which is a consortium of bacteria embedded in a self-produced polymer matrix composed of protein, polysaccharide, and DNA. In general, bacteria associated with biofilms are much more difficult to kill and remove [37]. *Pseudomonas aeruginosa* is highly resistant to disinfectants as well as antibiotics and is considered as one of the most problematic bacteria in healthcare facilities, and is responsible for approximately 10-20 % of hospital-associated infections [38]. The bacterium is naturally resistant to many antibiotics and disinfectants as a result of the permeability barrier from its Gram-negative outer membrane. Once the biofilms are formed, they are difficult to remove because the extracellular polymeric substance (EPS) is firmly attached to the surface and can block access of antimicrobial agents to individual cells, leaving behind a source for recontamination [39]. In general, *Pseudomonas aeruginosa* is referred to as one of the most problematic bacteria in healthcare facilities and is responsible for approximately 10-20 % of hospital-associated infections [38]. Chronic infections are among the significant concerns of bacterial biofilms as they are characterized by high tolerance to antibiotics and disinfectants as well as resisting phagocytosis and other components of the human body's defense system [40].

Although the general lowest removal efficiency (99.84%) from the final effluent was observed from the *Pseudomonas aeruginosa* microbial count, the EC treatment unit was able to lower the count from an average of 3197 to 31 CFU/100ml, which is equivalent to 99% removal efficiency. This means the combination of UF and UV radiation did not seem to be that effective for the elimination of *Pseudomonas aeruginosa*.

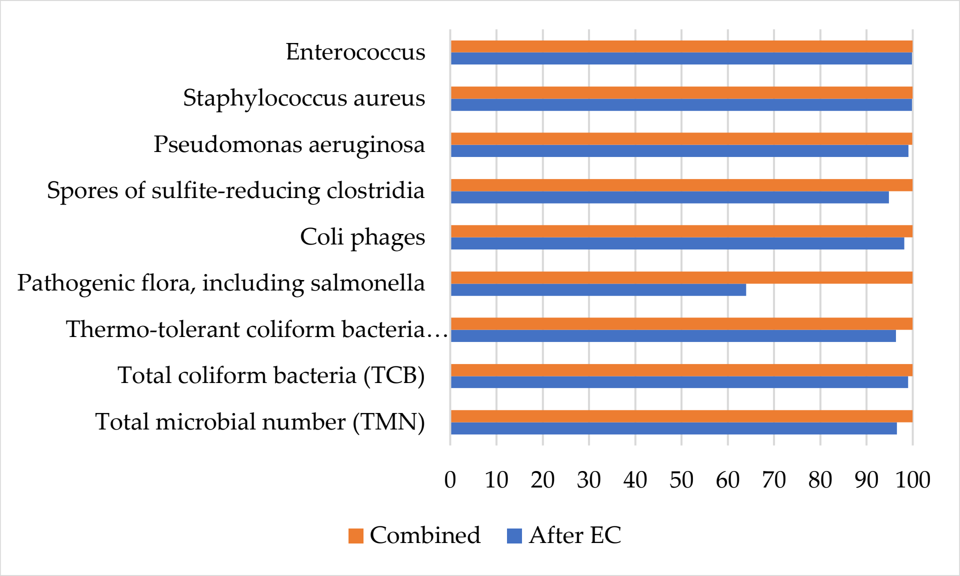

Figure 5. Microbes removal efficiency from EC and combined treatment (final) effluents

4. Conclusions

The applicability potential of an integrated treatment plant with EC, UF, and UV radiation for microbial elimination from poultry slaughterhouse wastewater has been studied. From the analysis results, it was observed that the EC treatment unit was able to remove the majority of the microorganisms with efficiency ranging from 63.95% to 99.83%. While on average 100% removal efficiency was achieved from most of the studied microbial parameters after the combined treatment with an exception of *Pseudomonas aeruginosa* that was still detected in the final effluent of some of the experimental sessions. Furthermore, the study observed that the EC treatment unit was more effective in the inactivation of *Pseudomonas aeruginosa* than the combination of UF and UV. In that matter, to eliminate the highly resistant microbial parameter, some upgrades to the treatment plant such as a general increase in the hydraulic retention time especially for the EC treatment unit would be required. It is of significant interest in the future to investigate the optimal conditions for 100% elimination of *Pseudomonas aeruginosa* from the poultry slaughterhouse wastewater. This study reveals further the potential of combining EC, UF, and UV radiation for a high-efficiency microbial elimination from poultry slaughterhouse wastewater.

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