STUDY ON THE SYNERGISTIC EFFECT OF MICROBIAL FUEL CELL ON THE TREATMENT OF LIVESTOCK AND POULTRY MANURE SLURRY

LIU, J. H.[1] – CHEN, D.[1] – MAHMOOD, A.[2*] – YANG, S. J.[1*]

[1]*Yantai Institute, China Agricultural University, 264670 Yantai, Shandong Province, China*

[2]*Department of Agronomy, University of Agriculture Faisalabad, Faisalabad, Pakistan*

[*]*Corresponding authors*
e-mail: athar.mahmood@uaf.edu.pk; sjyang-2008@163.com; phone: +86-153-3638-3005

(Received 22nd Feb 2024; accepted 13th Jun 2024)

Abstract. This study used a Microbial Fuel Cell (MFC) to treat livestock manure water. The objective was to explore the impact of MFC on the treatment of dairy farming manure water, while generating electricity. This study offers new insights into the resource utilization of livestock manure water and its compliance with discharge standards. A single-compartment membrane-free air cathode MFC was constructed in this study. The cathode material used was a flat plate cathode. This study employed anaerobic acidification of pretreated cow manure as the inoculum and used cow manure as the substrate to assess electricity production performance. Similarly, the degradation effect of the MFC on cow manure was also investigated. The results showed that the removal rate of Chemical Oxygen Demand (COD), ammonia nitrogen, suspended solids, nitrogen, phosphorus and potassium elements from cattle manure water by MFC reached 61.24%, 87.37%, 54.30%, 61.30%, 18.30% and 3.28%, respectively, with the maximal output voltage of 60.31 mV, and the maximal power density of 1.734 mW/m^3 (1 kΩ) within 24 h. As a result, the acidification pre-treatment microbial fuel cell effectively removed COD, ammonia nitrogen, suspended solids, and nitrogen elements.
Keywords: *livestock and poultry manure, pollutants, purification, microorganisms, electricity production*

Introduction

In China, the rapid expansion of livestock and poultry farming has led to massive poultry manure slurry production, all while meeting the demand for meat, eggs, and milk among the population. Practical experiences, both domestically and internationally, have demonstrated that using poultry manure slurry as soil fertilizer is the most cost-effective and efficient way to manage dairy waste. Usually, composting is an effective management of the solid waste. However, livestock manure effluent, comprising more than half the total amount of poultry manure slurry, has emerged as a substantial challenge to the sustainable development of livestock and poultry farming. Its large volume, low economic value, and impracticability for long-distance transport present a bottleneck in the industry. Currently, the primary approach to poultry manure slurrypoultry manure slurry management includes anaerobic fermentation or natural storage to ensure safe utilization as a soil fertilizer. However, when the amount of a poultry manure slurry effluent in a region surpasses the carrying capacity of local farmland, biochemical processes become the preferred method to meet the discharge standards. This wastes nutrient resources and increases the cost of poultry manure slurry management. The critical problem of the livestock and poultry farming industry must be addressed to reduce the management cost of manure discharge.

Currently, microbial fuel cell (MFC) technology primarily emphasizes the research and development of cathode and anode materials, proton exchange membranes,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37973813

microorganism screening and cultivation, biofilm curing, and reaction chamber design. The technology's future application prospects are centered on energy substitution, sensors, and sewage treatment. Chong (2012), Park and Zeikus (2003), and Cheng et al. (2006) investigated the power production performance of single-cell type MFCs. They used Fe^{3+}-doped graphite as the cathode, Mn^{4+}-doped graphite as the anode, and platinum-loaded carbon cloth as the cathode material and carbon paper as the anode material in their respective studies. The output power densities of the cells reached 788 mW/m2 and 1330 mW/m2, respectively. The primary challenge in developing MFCs as a novel energy source lies in enhancing both the output power density of the cell and the electron transfer rate from the electrodes. Studies on BOD (Biochemical Oxygen Demand) sensors have gained prominence due to the linear relationship between the current and the pollutant concentration of MFC cells.

Furthermore, the rapid and reproducible response of the current to the changes in effluent concentrations has further spurred research. While the MFC cell can shorten the BOD5 detection time, it produces a small current output signal when used as a sensor for nutrient-poor effluents. This happens primarily due to the significant diffusion rate of O2 through the cathode and the proton exchange membrane and a low rate of reduction at the cathode. These factors collectively affect the accuracy of the detection results. The main objectives of MFC research are to utilize organic wastewater as a fuel source and to harness chemical energy from the organic matter present in dairy effluents. Lu et al. (2009) used a single-chamber MFC to generate starch production wastewater and power. The system produced a maximum power density of 239.4 mW/m2 while concurrently removing up to 98% of the Chemical Oxygen Demand (COD) from the wastewater. There have been relatively fewer studies on the utilization of MFC for the treatment of livestock manure. Ni et al. (2020) utilized a dual-chamber microbial fuel cell to treat swine wastewater with a maximum output voltage of 0.61 V, a maximum power density of 770.97 mW/m2, and a COD removal rate of up to 64.36%. Syed et al. (2021) achieved an 80% COD removal, a maximum output voltage of 0.65 V, and a maximum power density of 12.75 mW/m2 from cow dung by using a single-chamber microbial fuel cell under ultrasonic pre-treatment conditions.

The type and utilization efficiency of a substrate, which serves as the source of energy conversion in MFC, significantly impacts the community structure and growth rate of microorganisms. It is also a critical factor in determining the efficiency of power production. Summarizing the results of previous studies, the focus of the study is to improve the power production efficiency of MFCs while simultaneously studying the degradation rate of the substrate, specifically, the degradation rate of COD. According to the requirements of China's "Discharge standard of pollutants for livestock and poultry breeding GB-18596" (China, 2001), COD or BOD5 serves as the vital control indicator for regulating pollutant discharge concentrations in wastewater. However, concerning water utilization from livestock manure, COD or BOD5 in manure effluent is a valuable source of soil organic matter. The agricultural industry standard "Technical Code of Practice for land application of livestock and poultry slurry manure NY/T-4046" (China, 2021) does not make specific limit requirements for the content of pollutants in manure effluent. It pays more attention to whether the quality of manure effluent meets crop growth needs and whether it is returned to the field to produce new pollution in the soil. Despite the clear advantages of MFC technology in recovering energy from wastewater, there has been limited research regarding its application in treating poultry manure slurry and evaluating its effectiveness from the perspective of wastewater discharge and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
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resource utilization. Hence, this study explores the treatment efficacy of MFC technology on dairy manure effluent while simultaneously generating electricity.

There are obvious differences between poultry manure slurry water and wastewater from other industries in terms of substrate composition, organic matter content, microbial species and quantity (Qiu et al., 2021). Different types of poultry manure slurry water also have different physical and chemical properties, which directly affect the MFC's electricity production performance and pollutant removal efficiency. Jeon et al. (2016) used SMFC to treat pig manure water, with a COD feed concentration of 4000 mg/L and a hydraulic residence time of 1 day, the COD removal rate reached 44%; Lin et al. (2014) research results on the use of SMFC to treat cow manure water showed that the COD feed concentration was 3228 mg/L, and the TP feed concentration was 113 mg/L. The hydraulic residence time is 1 day, the COD removal rate is only 20%, and the TP removal rate is 72% (Lin et al., 2014).This study aims to offer fresh insights into the resource utilization of poultry manure slurry effluents and to ensure compliance with discharge standards.

Material and Methods

MFC construction

The MFC in this experiment is an air-cathode single-compartment cell consisting of an anode and a cathode (*Fig. 1*). The reactor is made of an acrylic plate with a length, width, and height of 20 cm × 20 cm × 10 cm, respectively. The interior of the reactor was divided into eight anode chambers of 5 cm × 5 cm × 10 cm using a partition, with 5 cm diameter circular holes on the outer side of each anode chamber and 1 cm diameter holes between the anode chambers to facilitate the flow of slurry water. The cathode is placed on the outside of the anode chamber. The cathode / anode of each anode chamber was connected in series with the adjacent anode chamber to form a closed circuit. The anode is a carbon fiber brush connected with titanium wire, with a brush length of 6 cm, a bristle diameter of 3 cm, and a specific surface area of about 4500 m^2/m^3. The cathode is a roll-pressing air cathode, which is improved according to the method provided by Jiang et al. (2019), in which the conductive graphite mixed with polytetrafluoroethylene is sintered into the diffusion layer by heating in a water bath. The catalytic layer is made of capacitive-activated carbon and polytetrafluoroethylene, which is supported by the titanium wire mesh and pressed onto the titanium wire mesh by a roller to press the catalytic layer and diffusion layer together. The catalytic and diffusion layers were pushed onto the titanium wire mesh using a roller press, and the titanium wire on the titanium wire mesh was connected to a circuit (1000 Ω external resistor).

Pre-treatment of slurry water

Slurry water was taken from a dairy farm in Muping District, Yantai City, Shandong Province, and the physical and chemical properties of fecal water are shown in *Table 1*.

To prepare the sample, a 1000 mL conical flask was washed, dried, and covered with a silicone plug equipped with air conduit. The air conduit was connected with the air collection bag. 800 mL of cattle manure water was carefully transferred to the conical flask. The nitrogen gas was used to purge the air from the flask, after which the flask was promptly sealed. The conical flask was subsequently placed in a water bath 35℃ for

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
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acidification pre-treatment. The gas collection bag was monitored every 24 h, with the gas collected in the bag indicating the completion of the acidification pre-treatment.

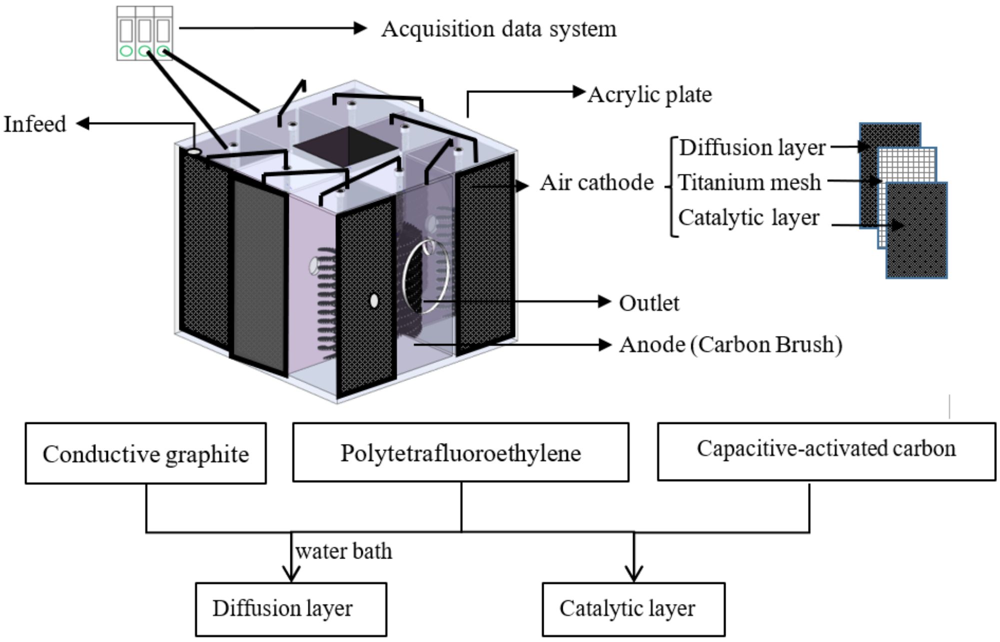

Figure 1. *MFC structure diagram and cathode procedural setup*

Table 1. *Physicochemical properties of cattle manure water*

Name	Value	Name	Value
TS (%)	3.22±0.05	COD (mg/L)	2456±13
TN (mg/L)	1025.70±18.71	EC (mS/cm)	7.61±0.15
TP (mg/L)	624.81±12.01	pH	7.46±0.1
TK (mg/L)	647.9±11.48	*Fecal coliform* (MPN/L)	$5.9 \pm 0.1 \times 10^6$
Ammonia nitrogen (mg/L)	500.87±15.71	*Ascaris lumbricoides egg* mortality rate (%)	11.3±2
SS (‰)	0.26±0.04		

Microbial fuel cell startup and operating conditions

At room temperature, the supernatant of the acidified fecal water was mixed with the kitchen waste digestate (from the laboratory, pH = 4.57) at a ratio of 4:1. The mixture was then slowly added into the anode chamber. The pH was carefully adjusted in the range of 5.5 ~ 6.5 using a 0.01 mol/L dilute sulfuric acid until the mixture level was approximately 1 cm below the upper surface of the anode chamber. The circuit was connected to initiate the MFC, and the initial output voltage was recorded. One cycle was completed once the MFC output voltage equaled or fell below the initial output voltage value. Subsequently, 1000 mL (approximately 50% of the adequate volume of the anode chamber) was discharged, and an equivalent volume of cattle manure water was replenished for the second cycle. After two cycles of stable operation, the freshly discharged liquid was placed in a water bath at 35°C for 24 h. Then, the supernatant of acidified manure water, kitchen waste digestate, and freshly discharged liquid were mixed

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at 4:1:1. The mixture was reintroduced into the anode chamber. Once the output voltage had stabilized or displayed regular fluctuations, the MFC successfully entered the operational stage. The material feeding and discharging occurred every 24 hours during the MFC operation. The volume of the removed material was $1/4^{th}$ of the anode chamber's optimal capacity, and the discharged material was promptly replaced with cattle manure water.

Sample collection and measurements

During each discharge, 100 mL from the discharge solution was used for testing, and the rest of the solution was adjusted to pH < 2 with 10% H_2SO_4 solution and stored at 4℃ in the refrigerator for spare use. The COD was determined using the closed reflux method (Naik and Jujjavarappu, 2020), while ammonia nitrogen was determined by the Nano-reagent colorimetric method (Qiao et al., 2023). Total nitrogen, phosphorus, and potassium were determined by the Kjeldahl method, molybdenum-antimony spectrophotometry, and flame photometry, respectively (Zhu et al., 2022). The pH is determined by the glass electrode method (China, 2021); conductivity (Electrical Conductivity-EC) by the conductivity meter method (Ren et al., 2014); suspended solids by the gravimetric method (Apha, 1998); *fecal coliform* counts by filter membrane method (Qiao et al., 2023); and *ascaris lumbricoides* egg mortality by centrifugal sedimentation method (Liang et al., 2020).

Automatic data collection and calculation

To facilitate data collection and recording, the cathode and anode of the MFC reactor were connected to a data acquisition card connected to a computer that displayed the measured voltage in real-time. The data acquisition card was provided by Beijing Qichengmofei Electronic Technology Co., Ltd, model number MPS-010602, and the detection frequency was 60 times/h. Current power density and Coulombic efficiency were calculated according to *Eq. 1, Eq. 2, and Eq. 3,* respectively (Logan et al., 2006).

$$I_{An} = \frac{U}{R_{Ex} \times V_{An}} \quad \text{(Eq.1)}$$

$$P = \frac{U^2}{R_{Ex} \times V_{An}} \quad \text{(Eq.2)}$$

$$CE = \frac{M\int Idt}{\Delta COD \times F \times b \times V_{An}} \times 100\% \quad \text{(Eq.3)}$$

The meaning of each symbol in the formula is as follows,

U — Battery output voltage, in millivolts (mV);

R_{Ex} — External resistor resistance value in ohms（Ω）

V_{An} — Effective volume of the anode chamber in cubic meters（m^3）

P — Power density in milliwatts per cubic meter（mW/m^3）

I_{An} — Current density in milliamps per cubic meter（mA/m^3）

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CE	—	Coulombic efficiency in percent（%）
M	—	Molecular mass of oxygen,32 g /mol
b	—	Number of electrons exchanged per mole of oxygen, $4e^-$
I	—	Current in milliamps（A）
F	—	faraday constant,96485 C/mol e^-
ΔCOD	—	The amount of COD removed from the MFC during a single run time, in milligrams per liter（mg/L)
t	—	Single run time in seconds (s): The single run time is typically 86400 s.

MFC polarization curve drawing

The MFC, which was stably running, was disconnected, and the open-circuit voltage value (E) was recorded. Subsequently, the external resistances were set to 11000 Ω, 2000 Ω, 1100 Ω, and 1010 Ω, respectively. Each resistance setting was run for two hours, and the process was repeated three times. The average voltage and current values were recorded under different resistances. The polarization curves were plotted with current density on the horizontal axis and voltage and power density on the vertical axis.

Statistical data analysis

Data were statistically analyzed using SPSS 25 and EXCEL 2021, and significant differences were handled using the Least Significant Difference (LSD) method.

Results and Analysis

Effect of MFC on COD removal from cattle manure slurry

Fig. 2 shows that the first discharge, i.e., the discharge after 24 hours of MFC operation, has the highest COD content but the lowest COD removal rate. With the increase in the number of discharges times, the COD content of the discharges showed a decreasing trend, but the COD removal rate increased accordingly. The COD removal rate of the 2nd, 3rd, 4th, and 5th discharges increased by 0.60%, 1.09%, 2.03%, and 2.11%, respectively as compared to the 1st discharge. The data showed that the highest COD removal rate was achieved in the 4th discharge. Still, the ANOVA indicated no significant difference ($P > 0.05$) between the COD removal rates of the 4th and 5th discharges, indicating that the system's operation was stabilized.

Effect of MFC on ammonia and nitrogen content of cattle manure slurry

The trend of ammonia nitrogen in the MFC-treated fecal slurry was like that of COD (*Fig. 3*). The ammonia nitrogen concentration in the feed was 500.87 mg/L, and the maximum ammonia nitrogen concentration in the discharge appeared for the first time. With the increase in discharges, the ammonia nitrogen concentration gradually decreased, and the ammonia nitrogen concentration in the fifth discharge was 35.17% lower than that in the first discharge. Contrary to the trend of ammonia concentration in the discharge, the ammonia removal rate increased with the number of discharges. The ammonia removal rate in the 5th discharge reached 1.09 times that in the first discharge. However, there was no significant difference in the ammonia removal rate in the 4th discharge ($P > 0.05$).

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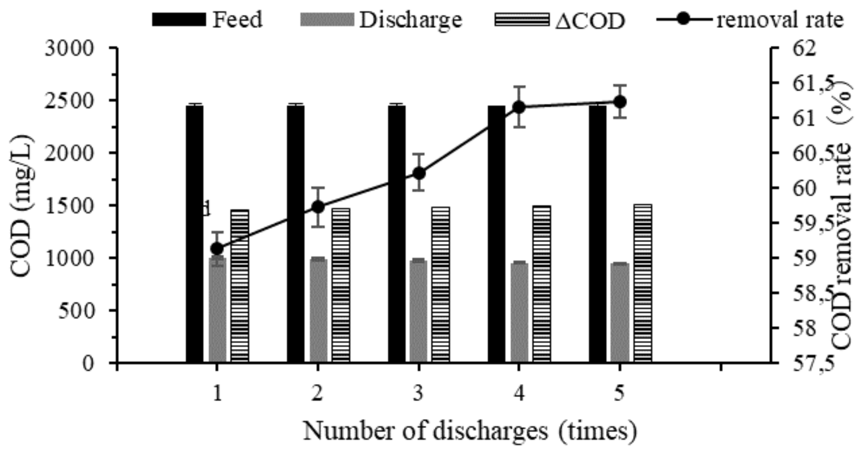

Figure 2. *Variation in COD removal from MFC-treated cattle manure slurry (LSD). Note: Differences in lowercase letters in the removal rate indicate significant differences (P < 0.05), while the same difference indicates non-significant differences (P > 0.05). COD removal rate = (Feed COD-Discharge COD) /Feed COD*

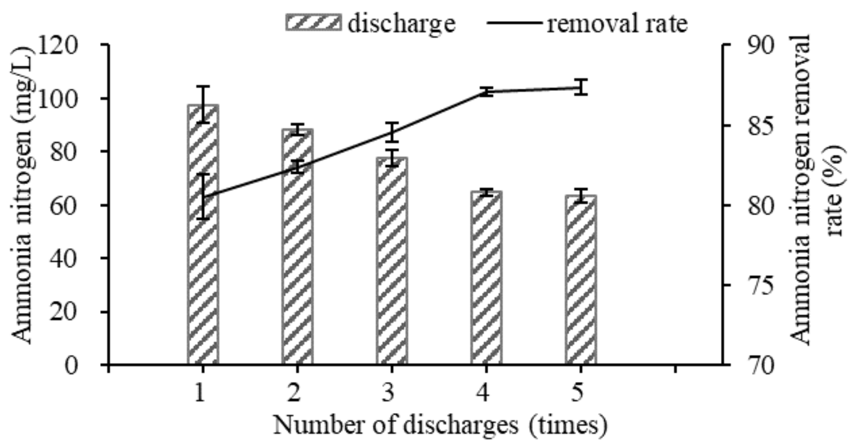

Figure 3. *Variation of ammonia nitrogen removal in MFC-treated cattle manure slurry (LSD). Note: Differences in lowercase letters in the same series indicate significant differences (P < 0.05), and the same shows non-significant differences (P > 0.05)*

Effect of MFC on suspended solids content in cattle manure slurry

The trend of suspended solids in the MFC-treated fecal slurry was like ammonia nitrogen and COD (*Fig. 4*). The concentration of suspended solids in the feed was 26380 mg/L. The maximum concentration of suspended solids in the discharge appeared for the first time. With the increased number of discharges, the concentration of suspended solids gradually decreased. The concentration of suspended solids in the 5th discharge was the lowest (54.30% lower than that of the feed). Analysis of variance (ANOVA) showed that there was no significant difference (P > 0.05) between the suspended solids removal rate of the 4th and 5th discharges, indicating that the operation of the system was stabilized.

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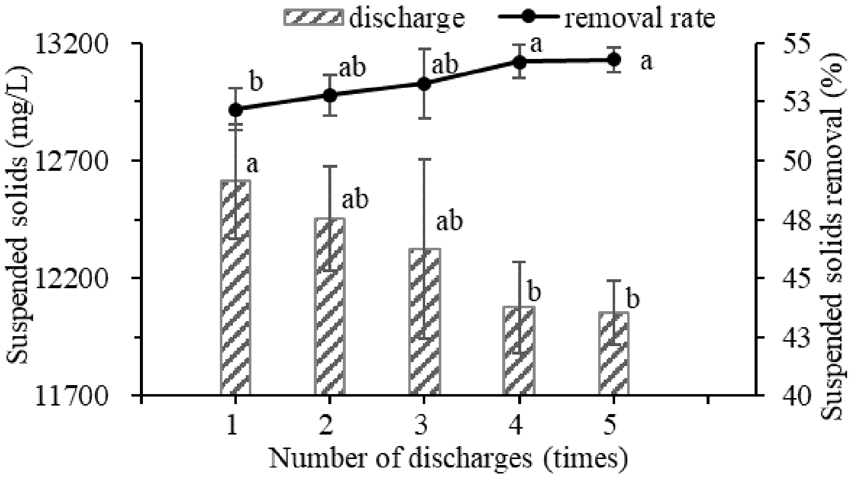

Figure 4. *Variation of suspended solids removal in MFC-treated cattle manure slurry (LSD). Note: Different lowercase letters on the shoulder labels of the same series of data indicate that the difference is significant (P <0.05), and the same suggests that the difference is not significant (P > 0.05)*

Effect of MFC on the EC of cattle manure slurry

The EC of the MFC feed was 7.61 mS/cm, and the EC values of the 1st, 2nd, and 3rd discharges showed a decreasing trend (*Fig. 5*). The EC values of the 1st, 2nd, and 3rd discharges decreased by 15.90%, 17.21%, and 18.79%, respectively, compared to the EC values of the feed. Unlike the first three discharges, the 4th discharge's EC value increased slightly, but the 5th discharge's EC value then decreased. The statistical analysis of the data showed that there was no significant difference between the EC values of the 3rd, 4th, and 5th discharges (*P > 0.05*).

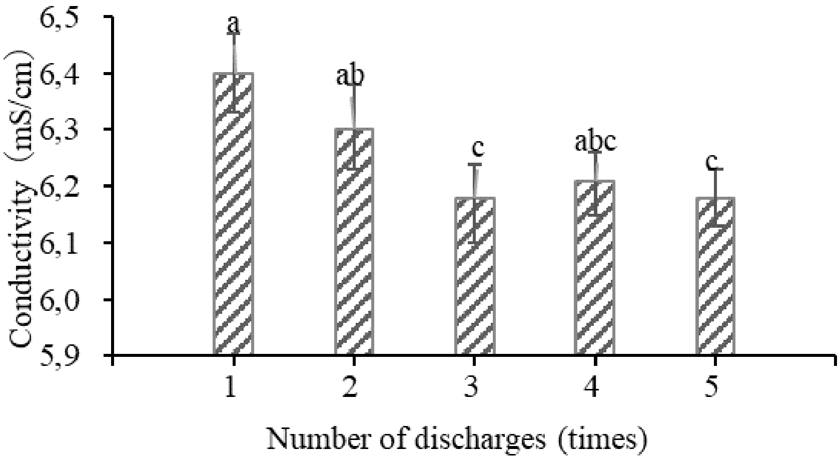

Figure 5. *Changes in EC of cattle manure slurry after MFC treatment (LSD). Note: Different lowercase letters on the shoulder labels of the same series of data indicate that the difference is significant (P <0.05), and the same suggests that the difference is not significant (P > 0.05)*

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Effect of MFC on nitrogen, phosphorus, and potassium content of cattle manure slurry

The first discharge, i.e., the discharge of the MFC running for 24 hours, has the highest elemental nitrogen content, indicating the lowest nitrogen removal rate. With the increase in discharges, the nitrogen content in the discharged liquid gradually decreased, while the nitrogen removal rate increased accordingly. Compared with the first discharge, the nitrogen removal rate in the 2nd, 3rd, 4th, and 5th discharges increased by 2.73%, 3.15%, 5.72%, and 6.05%, respectively. The data showed that the highest nitrogen removal in the 5th discharge was achieved. Still, the ANOVA indicated no significant difference in nitrogen removal between the 4th and 5th discharges (*Table 2*).

Table 2. *Changes in nitrogen, phosphorus, and potassium content of MFC-treated cattle manure slurry (LSD)*

Chemical element	Content (mg/L)					
	Infeed	Discharge 1	Discharge 2	Discharge 3	Discharge 4	Discharge 5
N	1025.70±18.71	459.05 ^{a}±11.91	431.00 ^{b}±14.64	426.70 ^{b}±12.14	400.39 ^{c}±11.38	396.97 ^{c}±10.38
P	624.81±2.01	446.76 ^{d}±9.29	459.62 ^{c}±2.79	471.20 ^{b}±3.46	503.06 ^{a}±5.91	510.44 ^{a}±6.55
K	647.90±	618.90 ^{b}±2.13	623.83 ^{ab}±4.82	626.46 ^{a}±3.21	626.32^{ab}±4.46	626.64 ^{a}±4.53

Note: Peer data with different shoulder-marked lowercase letters indicate significant differences (P < 0.05), and the same indicates non-significant differences (P> 0.05)

The lowest phosphorus content was found in the effluent of the MFC running for 24 hours, but the highest phosphorus removal rate of 18.30% was found in the effluent. With the increase in the number of discharges, the phosphorus content in the discharges showed a gradually increasing trend, and the phosphorus removal rate decreased; compared with the removal rate of the first discharge, the phosphorus removal rate in the discharged liquid of the 2nd, 3rd, 4th, and 5th discharges decreased by 2.06%, 3.91%, 9.01%, and 10.19%, respectively. The analysis of variance (ANOVA) showed no significant difference in the phosphorus removal rate of the 3rd, 4th, and 5th discharges, indicating that the system's operation was stabilized.

The lowest value of potassium concentration in the discharge appeared the first time, and with the increase in discharges, the potassium concentration gradually increased. The potassium concentration in the 5th discharge time was 1.25% higher than the first discharge. In contrast to the trend of potassium concentration in the discharge, the potassium removal rate decreased with the increase in the number of discharges. The potassium removal rate in the 5th discharge was 96.72% of that in the first discharge. The analysis of variance (ANOVA) indicated no significant difference in the potassium removal rates of the 3rd, 4th, and 5th discharge.

Effect of MFC on Escherichia coli content and mortality of Ascaris lumbricoides eggs in cattle manure slurry

The *fecal E. coli* counts of all five discharges showed a decreasing trend. The *fecal E. coli* counts in the five discharges decreased by 9.83%, 10.17%, 10.51%, 11.02%, and 11.69%, respectively, compared to the *fecal E. coli* counts in the feed. Although the lowest *fecal E. coli* count was found in the 5th discharge, there was no significant difference from the first four discharges. Unlike *fecal coliforms*, ascarid egg mortality in the discharge gradually increased, but there were no significant differences between the five discharges (*Table 3*).

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DOI: http://dx.doi.org/10.15666/aeer/2204_37973813

Table 3. *Changes in fecal coliform counts and Ascaris lumbricoides egg mortality in MFC-treated cattle manure slurry (LSD)*

Number of discharges (times)	Number of fecal coliform bacteria（×106 MNP/L）	Ascaris lumbricoides egg mortality rate（%）
1	5.32^{a}±0.09	20.67^{a}±0.15
2	5.30^{a}±0.05	20.74^{a}±0.6
3	5.28^{a}±0.08	20.90^{a}±0.6
4	5.25^{a}±0.06	21.20^{a}±0.8
5	5.21^{a}±0.05	21.46^{a}±0.37

Note: Differences in lowercase letters in the same column indicate significant differences (P < 0.05), while the same difference indicates non-significant differences (P> 0.05)

Power generation performance of MFC

During 0~10 h after the 1st feeding, the voltage was stabilized within the range of 48 mV±3 mV, then the voltage began to increase, and by 13 h after feeding, the voltage increased to 60.31 mV, which was 20.31% higher than that of 10 h after feeding. At 13~24 h after feeding, the voltage showed fluctuations, and by 24 h after feeding, the voltage dropped to 40.79 mV, which was lower than the voltage at the start of feeding. After the 2nd feeding, the voltage was maintained in the range of 50 ±4 mV. After the 3rd feeding, the voltage was maintained in the range of 48 ±4 mV, and the voltage tended to be in a stable state and maintained in the range of 48 ±3 mV after the 4th and 5th feeds (*Fig. 6-A*).

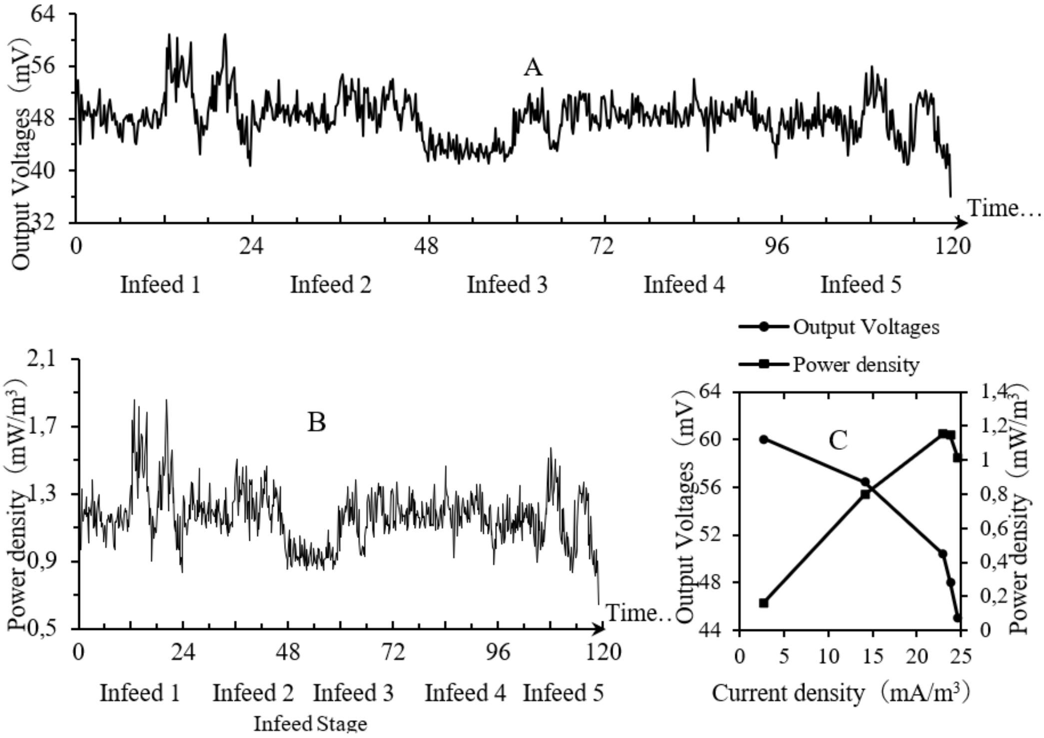

Figure 6. *The power generation performance of MFC. Note: A and B in the figure denote the voltage and power density of the MFC, respectively, and C represents the current density function*

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The trend of MFC power density at different feeding times was similar to that of voltage (*Fig. 6-B*). The power density variation within 24 h after the 1st feeding was significant, with multiple peaks in 12-15 h after feeding; the maximum power density was 1.734 mW/m^3 (1 kΩ), and the minimum power density was 0.832 mW/m^3. The power density variation during the 24 h after the 2nd and 3rd feeding gradually decreased, and then the power density was stabilized after the 4th and 5th feeding, showing periodic and regular changes.

As can be seen from the current density function (*Fig. 6-C*), the current density tends to decrease as the voltage increases, and there is a good negative correlation between the two. Unlike voltage, there is a positive correlation between power density and current density; as power density increases, current density increases accordingly.

Coulombic efficiency of MFC

The variation of Coulombic efficiency after MFC feeding is shown in *Fig. 7*. The maximum value of coulombic efficiency occurs at the 1st feed and decreases significantly with the increase in the number of feeds and falls to the minimum value at the 3rd feed. Compared with the 1st feed, the coulombic efficiency decreased by 9.66% after the 3rd feed. The coulometric efficiency increased significantly after the 4th feed but declined after the 5th.

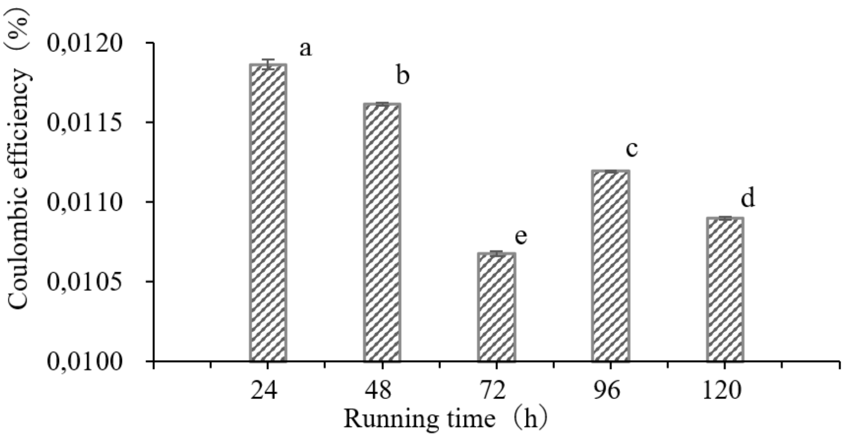

Figure 7. *Variation of Coulombic efficiency after MFC feeding* **(***LSD***).** *Note: Differences in lowercase letters in the same series indicate significant differences (P> 0.05), and the same shows non-significant differences (P> 0.05)*

Discussion

COD is an indicator that measures the concentration of reducing substances, particularly organic matter, in wastewater and, to a certain extent, reflects the mild degree of pollution in a water body. The results of the study demonstrated that MFC has the capability to effectively degrade COD in cattle manure slurry. Firstly, non-electrogenic bacteria break down the organic matter in cattle manure slurry through hydrolysis, acidification, and methanogenesis. Secondly, the electrogenic bacteria engage in life metabolism by consuming small-molecule organic matter. This metabolic activity results in the release of electrons and generation of electrical current(Logan and Regan, 2006). After the first three feeds, the COD content in the bovine manure slurry showed a

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DOI: http://dx.doi.org/10.15666/aeer/2204_37973813

continuous downward trend, and the COD removal rate increased accordingly (*Table 1*). The observed phenomena primarily result from the extended operation time of the MFC. Over time, the biofilm scale gradually expanded, creating more attachment points for microorganisms. Expanding the biofilm's surface area facilitated the number of reactions, attaching more microorganisms to the anode chamber. This led to a substantial increase in the microorganism population, which enhanced the COD removal rate.

Furthermore, there was significant competition among microorganisms at the initial feeding stages. However, as the cell's operation stabilized, a dominant biological community emerged, consisting of electrogenic and non-electrogenic bacteria that adapted to the reaction environment. This reduced the intensity of competition, fostering increased microbial activity and, consequently, an elevated COD removal rate. Following the fourth and fifth feeds, the COD removal rate in the cattle manure slurry remained relatively stable. This suggests that, when the feed composition remained relatively stable, factors such as the biofilm scale, microbial community, and population either remained stable or changed predictably until the MFC operation reached a steady state.

The removal of ammonia nitrogen from cattle manure slurry by MFC consists of two main pathways: physical elimination and chemical elimination. During power generation, ammonium ions are converted to the more volatile ammonia gas due to increased pH near the cathode (protons are consumed).At the same time, there is a nitrification/denitrification reaction process in the cathode of the MFC, which converts the NH_4^+ and NOx- in the cattle manure slurry into ammonia gas and nitric acid (Yang et al., 2021). Finding of the study demonstrated that the ammonia nitrogen removal rate of MFC for cattle manure slurry exhibited a gradual increase followed by stabilization during the initial feeding period (the first three cycles). This was primarily attributed to the growth of cathodic biofilm, leading to the increased attachment points for nitrifying bacteria and an enlarged responsive surface. The ammonia nitrogen absorption gradually increased during the nitrification reaction process (Jiang, 2019). As the MFC continued to operate steadily over numerous cycles, the biofilm structure gradually stabilized. The number of dominant flora and bacterial activity reached their maximum levels, and the rate of biological metabolism achieved equilibrium. Consequently, the processes of nitrification and denitrification reached saturation, leading to a gradual stabilization of the ammonia and nitrogen removal rates in the discharged cattle manure slurry.

The effect of MFC on suspended solids concentration in cattle manure slurry is closely related to biofilm adsorption and microbial metabolic activities. Biofilm adsorption, degradation, and absorption by microorganisms such as electrophiles and nitrifying bacteria are the main reasons for reducing suspended solids in cattle manure slurry (Ghosh et al., 2000). In the early feeding period (the first three cycles), the continuous growth of MFC biofilm strengthened the adsorption of suspended solids, which was the main reason for the gradual decrease of broken solids content in the discharged cattle manure slurry. With the gradual stabilization of the biofilm structure, the electron transfer on the electrodes was blocked, and the metabolic rate of the electrogenic bacteria decreased, hindering the whole reaction process. Eventually, the suspended solids removal rate was gradually stabilized (Jiang et al., 2022). The decrease in conductivity of cattle manure slurry is mainly caused by the change in ion concentration and the reduction of soluble compounds.

On the one hand, a large portion of free ammonia and nitrogen ions in the feed participated in nitrification/denitrification, ultimately converting into nitrogen. This conversion reduced the presence of free ions, resulting in a decrease in conductivity. On

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37973813

the other hand, soluble compounds, like VFAs (volatile fatty acids), were consumed in large quantities as a substrate for microbial electricity production. Consumption of these soluble compounds in the system reduced their concentration, thereby causing a decrease in the EC value (Racz and Fitzgerald, 2001).

MFC can reduce the nitrogen content in cattle manure slurry, mainly because of the loss of ammonia and nitrogen during the operation of MFC and the formation of insoluble precipitates from certain nitrogen and metal compounds during denitrification (Yokoyama et al., 2006). After the initial feeding (the first three cycles), the loss of ammonia and nitrogen is large, resulting in a significant decrease in nitrogen content. Then, the MFC operation is gradually stabilized, and there is no further decrease in the nitrogen content of cattle manure slurry. MFC technology also leads to a reduction in the phosphorus content of cattle manure slurry. This reduction primarily occurs because the suspended matter in cattle manure slurry undergoes adsorption by the carbon brushes and cathode biofilm, preventing its decomposition and subsequently facilitating adherence and settling (Lin et al., 2014). As the number of feeding cycles increased, the phosphorus removal rate gradually decreased and eventually stabilized. This decline was attributed to the slower growth rate of the biofilm, which resulted in the original biofilm reaching its adsorption saturation point. Additionally, the introduction of new feed solutions led to a reduction in the adsorption of suspended solids, contributing to the stabilization of the phosphorus removal rate. On the other hand, the gradual decomposition of suspended solids adsorbed in the biofilm may lead to a decline in the phosphorus and its removal rate (Lin et al., 2014) . Like phosphorus, MFC also reduces potassium content in cattle manure slurry due to the adsorption of suspended solids. Still, the effect on potassium content is relatively mild, with a maximum removal rate of potassium of only 4.48%, mainly due to the presence of potassium as a soluble ion in cattle manure slurry, which increases the EC to improve the transfer efficiency of electron exchange (Victoria and Mercy, 2020).

MFC can kill *E. faecalis* and *Ascaris lumbricoides eggs* in bovine manure slurry. However, the content of E. faecalis in the five discharges gradually decreased. As the mortality rate of *Ascaris lumbricoides eggs* gradually increased, the ANOVA showed no significant difference. This suggests that the biofilm had not yet reached saturation during the initial feeding period (the first two cycles). Oxygen diffusion was still occurring, and electron transfer was minimally obstructed. Electrogenic bacteria rapidly consumed organic acids, resulting in limited organic acid accumulation. Consequently, the inactivation effect on *E. faecalis* and *Ascaris lumbricoides eggs* was relatively weak during this phase. As the biofilm reached saturation, the rate of oxygen diffusion through the cathode into the anode chamber decreased progressively. Consequently, the concentration of organic acids increased, strengthening the inactivation effect on *E. faecalis* and *Ascaris lumbricoides eggs* (Rojas-Oropeza et al., 2017). Due to the small capacity of the MFC cells in the experimental design, there was limited accumulation of organic acids during power production. The concentration increase was insignificant, and the changes in *fecal E. coli* content and Ascaris lumbricoides egg mortality rate in the effluent were not substantial.

At the initial feeding phase (0~10 h), the concentration of small molecules in cattle manure slurry were low, which limited the metabolic rate of the electrogenic bacteria. At the same time, cattle manure slurry contains several strains that the system's directional selection has not selected. The electrogenic bacteria compete with these strains, which collectively leads to low activity of the electrogenic bacteria and low output voltage

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DOI: http://dx.doi.org/10.15666/aeer/2204_37973813

(Wang et al., 2013; Li et al., 2022). In the subsequent feeding cycles, a significant portion of the macromolecules undergo hydrolysis, resulting in ample substrate availability. This abundance of substrate fuels an increase in microbial activity, and the microbial community undergoes evolution, influenced by the system's directional selection. The electrogenic bacterial population takes over, resulting in a rise and stabilization of the output voltage (Chong, 2021). The power density variation of MFC is similar to that of voltage. During the initial feeding period (0-10 hours), the anode surface exhibited the attachment of not only a biofilm consisting of various electrochemically active microorganisms but also a substantial number of insoluble substances. The presence of these non-conductive substances on the anode surface had a significant impact on both the quantity and uniformity of electricity-producing microorganisms' attachment. Additionally, it influenced the transfer of electrons and protons in the solution, ultimately affecting the power output of the MFC (Jiang et al., 2022). As the concentration of suspended solids in cattle manure slurry decreases, and the biofilm structure is gradually improved, the amount of nonconductive material attached to the anode decreases accordingly. It remains relatively constant, resulting in a corresponding increase and stabilization of the power density.

Coulomb efficiency refers to the ratio of the MFC's discharge capacity to the charging capacity during the same cycle, which is affected by a variety of factors, among which the battery type and biofilm play a decisive role. Due to the diffusion of oxygen through the cathode, the Coulomb efficiency (CE) of a single-chamber MFC is lower than that of a two-chamber MFC. During the initial feeding period of the MFC, specifically in the first three cycles, a notable disparity exists in the quantity and activity of electrogenic bacteria versus non-electrogenic bacteria. During this period, non-electrogenic bacteria tend to dominate, resulting in lower current and consequently lower Coulombic efficiency (Karthikeyan et al., 2016). On the contrary, as the biofilm grows, the cathodic biofilm begins to impede the entry of oxygen into the catalytic layer, affecting the rate of electron exchange. Simultaneously, the attachment of non-conductive substances within the biofilm hinders the attachment and even distribution of electrogenic microorganisms on the anode. This leads to increased internal resistance and activation polarization, as well as ohmic polarization, all of which contribute to the decrease in the Coulombic efficiency of the MFC (Logan et al., 2006). According to the corresponding output voltage and power density, it is known that there is a significant decrease in voltage after the 3rd feeding, which is mainly due to the increase in anode oxygen concentration, resulting in a reduction of power production and Coulombic efficiency. At the same time, changes in microbial communities affect the electron discharge efficiency, thereby affecting the performance of MFC (Aelterman et al., 2006). There is also the possibility that Columbic is less efficient due to a variety of metabolic reactions and increased mass transfer loss (Xiao et al., 2013; Raghavulu et al., 2013).

The activity and properties of the biofilms formed on the electrodes are regulated by physical, chemical, biological, and electrochemical parameters. The degree of biofilm formation on the anode surface also affects the performance of MFC (Mohan et al., 2008). It has been proposed that during MFC operation, the substrate is available in the outer layer of the biofilm, while the electrode is only available in the inner layer of the biofilm, thereby forming a thin and open biofilm that allows the substrate to migrate without hindering the transfer of electrons to the electrode (Wang and Han, 2009). Although the formation of biofilms is essential for electron transfer and bioelectricity, the thick deposition of biofilms hinders the flow of electrons (Mohan et al., 2014). The reason for

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the lower Pmax produced by Lin (2014) and Yokoyama et al. (2006) may be that the wastewater from pig farms and the wastewater from cattle farms treated by the two contain a large amount of suspended manure, resulting in a layer of biological membrane composed of a variety of electrochemically active microorganisms attached to the anode surface, but also a large amount of non-toxic suspended manure. Soluble substances (feces), the attachment of these non-conductive substances to the anode greatly affects the adhesion, uniformity, and transfer of electrons and protons in the solution of electricity-producing microorganisms, resulting in higher internal resistance and activation polarization and ohmic polarization, which in turn affects the MFC power output.

Conclusion

Present findings demonstrate that MFC has a significant removal effect on COD, ammonia nitrogen, suspended solids, nitrogen, phosphorus, and potassium elements in cattle manure water, and the removal rate reached to 61.24%, 87.37%, 54.30%, 61.30%, 18.30%, and 3.28%, respectively. MFC can significantly reduce the EC value of cattle manure water, but the inactivation of *fecal coliforms* and *Ascaris lumbricoides eggs* was insignificant. MFC-treated cattle manure water meets the relevant requirements of the “Technical code of practice for land application of livestock and poultry slurry manure” (NY/T 4046-2021), but it has not yet met the emission requirements of “Discharge standard of pollutants for livestock and poultry breeding” (GB 18596-2001), which NH_4^+-N less than 80 mg/L and COD less than 400 mg/L.

Acknowledgements. This work was supported by the Yantai Science, Technology and Innovation Development Project (2023JCYJ096), Yantai Integrated School-Site Development Project (2023XDRHXMPT12) and Construction Project of Experimental Center of Yantai Institute, China Agriculture University.

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