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Supplement of

Cr(VI) reduction, electricity production, and microbial resistance variation in paddy soil under microbial fuel cell operation

Huan Niu et al.

Correspondence to: Can Wang (wangcan@swjtu.edu.cn)

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Supplement

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S1: Preparation of cathode supported by Fe_3O_4 catalyst

Cathode preparation: 100.00×50.00×3.00 mm graphite felt (GF) (purchased from Jiangsu Xinye Electronic Materials Factory, Jiangsu, China) was selected as the cathode material of the SMFC, which was ultrasonicated in ethanol for 50 min to remove impurities, removed, and washed. Then put it into an oven and dried at 60℃ for 12 h. It was put into the hydrothermal synthesis reactor and added concentrated nitric acid, and reacted at 90℃ for 9 h. After the reaction was completed, the GF was rinsed continuously with ultrapure water to ensure that the pH of the rinsed water was neutral, and dried at 60℃ for 12 h to complete the pre-activation of the GF. The Fe_3O_4 catalyst was obtained by dissolving 3.60 g of ferric chloride hexahydrate, 6.00 g of sodium acetate, and 1.00 g of sodium citrate in 140 ml of ethylene glycol, and repeatedly ultrasonicating for 2 h to form a homogeneous solution. The pre-activated GF was put into a hydrothermal synthesis reactor: the Fe_3O_4 catalyst was added and soaked for half an hour and then heated at 200℃ for 8 h. After heating, the reactor was cooled down to room temperature, washed repeatedly with ultrapure water and ethanol, and then put into an oven at 60℃ to dry for 12 h. The reaction was then dried at 60℃.

S2: Python code of Raspberry Pi voltage acquisition system

```
# -*- coding:UTF-8 -*-
from threading import Timer
import time
import RPi.GPIO as GPIO
import datetime
import os
import sys
import xlwt

sys.path.append('./modules/')

from GetVoltage import get_voltage
from modules.ADS1263 import ADS1263

GPIO.setmode(GPIO.BCM)
CollectTimes = 150

def _get_average_list():
    """smooth"""
    if CollectTimes <= 0:
        print("Error number for the array to averaging!/n")
        return -1
    elif CollectTimes <= 5:
        return sum(VoltageArray) / CollectTimes
    else:
        return sum(VoltageArray) / CollectTimes

# clean txt
book = xlwt.Workbook(encoding='utf-8',style_compression=0)
sheet = book.add_sheet('MFCdata',cell_overwrite_ok=True)
col = ('current time','MFC1','MFC2','MFC3')
for i in range(0,4):
    sheet.write(0,i,col[i])

x=0
while(1):
    x=x+1

    time.sleep(1)

    VoltageArray = []
```

```
    for i in range(CollectTimes):
        VoltageArray.append(get_voltage(0))
    adc1 = _get_average_list()

    ############################################################

    VoltageArray = []
    for i in range(CollectTimes):
        VoltageArray.append(get_voltage(1))
    adc2 = _get_average_list()

    ############################################################

    VoltageArray = []
    for i in range(CollectTimes):
        VoltageArray.append(get_voltage(2))
    adc3 = _get_average_list()

    ############################################################

    curr_time = datetime.datetime.now()
    time_str = datetime.datetime.strftime(curr_time,'%Y-%m-%d %H:%M:%S')

    datalist = [time_str,str(adc1),str(adc2),str(adc3 )]
    print(datalist)

    for j in range(0, 4):
        sheet.write(x, j, datalist[j])
        savepath = '/home/pi/excel.xls'
        book.save(savepath)

time.sleep(598.5)
```

S3: Sampling method

We used a plastic cylindrical straw with a diameter of 0.40 cm and a length of 16.00 cm as the sediment sampler. The SMFC sediment part is inserted by the sampler vertically at a specific time, and then quickly removed, and the upper, middle, and lower parts of the sampler are mixed as a determination sample. And the fresh sample each time is only 8.00-16.00 g, only 0.20-0.40% of the SMFC. The total sampling amount shall not exceed 5% of the total population. Because the sampler is much smaller than SMFC, the disturbance is avoided to a great extent and the normal operation of SMFC is guaranteed.

S4: Soil DNA extraction method

Microbial DNA Rapid extraction kit (Shenggong Bioengineering Co., LTD., Shanghai, China) was used to extract total DNA from fresh samples. Specifically, 0.50 g sample, 0.50 g magnetic beads, and 1.00 ml SLX-Mlus Buffer were added in a 2.00 ml Eppendorf tube, and ground for 250 s under 45 HZ. Then added and mixed with 100 μl DS Buffer, and cultivated under 70 ℃ for 10 min and then 90℃ for 2 min. Then the mixture was centrifuged at 10000 g for 5 min at room temperature. 800 μl supernatant was moved to a new tube and added with 270 μl P2 buffer and 100 μl HTR reagent, and then cultivated under -20 ℃ for 5 min and then centrifuged again at 10000 g for 5 min. The supernatant was then moved to a new 2 ml tube added with the same amount of XP5 buffer and mixed upside down for 8 min. After magnetic rack adsorption, discard the residual liquid, remove the tube, add 500 μL XP5 Buffer, and mix well. Then adsorbed again with a magnetic rack, discard the residual liquid, remove the tube, add 500 μL PHB, and mix well. Then adsorbed again with a magnetic rack, discard the residual liquid, remove the tube, add 500 μL SPW Wash Buffer, and mix well (repeat this step twice). Then the mixture was adsorbed again with a magnetic rack, discard the residual liquid, was centrifuged in the tube under 10000 g for 10 s. Then the beads were adsorbed again with a magnetic rack, discard the residual liquid, and let

stand for 8 min. After that, the beads were added with 100 μL elution buffer, mixed, and let stand for 5 min. Finally, after adsorbing with a magnetic rack, the supernatant was moved to a new 1.00 ml Eppendorf tube, and total DNA was obtained for further use. The PCR reaction system was constructed.

Table S1 Main material dimensions

Material	Length/cm	Width/cm	Area/cm^2
Collector plate	10.50	5.50	57.75
Aluminum foam	6.60	5.40	35.64
GF	10.00	5.00	50.00

Table S2 Primer sequence of HRGs and MGEs

Gene	Primer sequence	Function description	Ref
chrA-F	TCCTTCGGCGGCCCTGCCGGNCARATHGC	*chrA* encodes a transporter protein involved in chromate efflux.	(Rivera et al., 2008)
chrA-R	GTAGGTGGCCAGCTGCTNGCYTCNGGNCC		
chrB-F	CCGGAATTCATGCGTGTCTGGCGAACCCTGA	*chrB* genes regulate the transcription of genes in the transporter protein complex.	(Branco and Morais, 2013)
chrB-R	CCCAAGCTTTCACTCTGCGGAAGAACGA		
ChrR-F	AGGAACTTCTGCGTGCCCTC	The chromate reductase chrR is the best-known of	(Nepple et al.,
ChrR-R	TACGGTGACAGTGCGTTTGC		

		the reductases that catalyze the reduction of Cr^{6+} to Cr^{3+}.	2000; Baldiris et al., 2018).
IntI-F	CGAACGAGTGGCGGAGGGTG		
IntI-R	TACCCGAGAGCTTGGCACCCA		
tnpA02-F	GGGCGGGTCGATTGAAA	MGEs such as integrons, plasmids, and transposons play a key role in the transfer of resistance genes between different microorganisms in the environment	(Wu et al., 2022; Wu et al., 2023).
tnpA02-R	GTGGGCGGGATCTGCTT		
tnpA05-F	GCCGCACTGTCGATTTTTATC		
tnpA05-R	GCGGGATCTGCCACTTCTT		

Table S3 Distribution percentage of EDS elements in electrode materials.

Elements	Samples					
	Cathode	Fe_3O_4-cathode	Cathode after operation	Anode	Anode with EAB-loading	Anode after operation
C	93.74%	66.85%	33.83%	6.70%	14.65%	14.35%
O	6.26%	19.97%	38.96%	47.57%	18.49%	50.92%
Fe	n.d.	13.17%	8.07%	n.d.	n.d.	0.88

Mg	n.d.	n.d.	0.44%	n.d.	0.41%	n.d.
Al	n.d.	n.d.	3.34%	37.04%	53.72%	17.56%
Cr	n.d.	n.d.	0.08%	n.d.	n.d.	0.12%
Cu	n.d.	n.d.	n.d.	n.d.	1.59%	n.d.
Zn	n.d.	n.d.	n.d.	n.d.	1.81%	n.d.
Si	n.d.	n.d.	7.16%	8.68%	9.32%	2.58%
Na	n.d.	n.d.	2.18%	n.d.	n.d.	4.55%
Ca	n.d.	n.d.	4.81%	n.d.	n.d.	n.d.
K	n.d.	n.d.	1.12%	n.d.	n.d.	0.58%
Cl	n.d.	n.d.	n.d.	n.d.	n.d.	2.67%
P	n.d.	n.d.	n.d.	n.d.	n.d.	5.81%

Table S4 SMFC power generation performance on 15-day and 30-day

Resistor (Ω)	15d-Current density (mA/m^2)	15d-Power density (mW/m^2)	30d-Current density (mA/m^2)	30d-Power density (mW/m^2)
51	448.57	37.43	485.23	42.21
100	386.53	53.84	424.57	63.47
200	296.55	62.24	357.26	90.22
510	202.88	73.52	238.08	102.02
1000	131.18	60.60	157.54	87.35
2000	75.99	40.43	95.91	64.58
5100	31.55	17.77	43.88	34.41
10000	17.01	10.13	23.95	20.09

Table S5 Performance comparison of various configurations of SMFC.

Reactor	configuration	Chamber volume (L)	Output voltage（V）	Maximum power density (mW/m^2)	Reference

Single-chamber SMFC	0.72	0.33	17.30	(Li et al., 2016)
Single-chamber SMFC	2.16	0.30	12.10	(Yu et al., 2017)
Two-chamber SMFC	4.20	0.40	29.78	(Srivastava et al., 2019)
Single-chamber circle SMFC	0.95	0.35	24.00	(Yu et al., 2021)
Single-chamber SMFC	8.00	0.40-0.60	0.20 mW	(Yoon et al., 2023)
Single-chamber circle SMFC	n.m.	n.m.	25.51	(Wang et al., 2023)
Single-chamber SMFC	9.70	n.m.	70.40±1.40	(Dhillon et al., 2023)
Two-chamber SMFC	0.05	n.m.	71.00 ± 0.82	(Zhang et al., 2023a)
Constructed wetland MFC	38.85	0.20	12.50	(Tao et al., 2023)
Constructed wetland MFC	4.08	0.43	28.10	(Niu et al., 2023)
Plant-SMFC	0.77	0.51	46.80	(V et al., 2023)
Single-chamber circle SMFC	2.00	0.17	10.00	(Youssef et al., 2023)
Constructed wetland MFC	2.70	0.39	4468.40	(Zhang et al., 2023b)
Two-chamber SMFC	0.50	0.31	20.35	(Zhang et al., 2024)
Constructed wetland MFC	13.00	0.17	1.40	(Dai et al., 2024)
Plant-SMFC	1.05	0.55	8957.70	(Chen et al., 2024)
Single-chamber circle SMFC	1.60	0.40	472.52±14.20	(Zhao et al., 2024)

Single-chamber circle SMFC	1.40	0.53	178.17	(Sun and Wang, 2024)
Single-chamber SMFC	2.30	0.75	102.00	This study

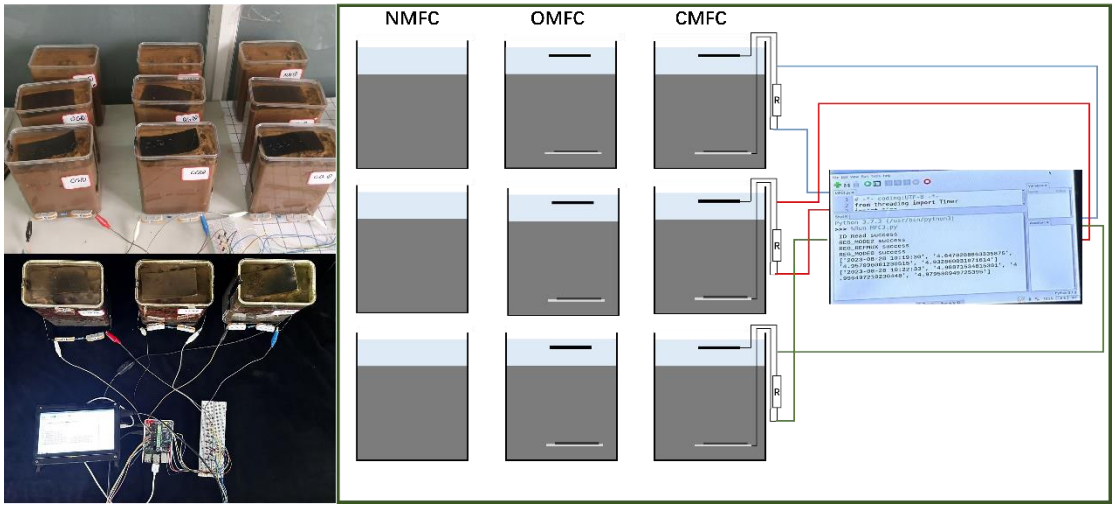


Fig. S1 SMFC structure and experimental grouping.

The left part of the picture is the SMFC photo, and the right part is the model groups. A plastic box (140.00×85.00×165.00 mm) was used as the SMFC reactor, with 1.50 kg soil and overlying water of 3.00 cm to simulate the flooded state during rice planting. The cathode was floated on the water surface while the anode was buried (about 3.00 cm from the bottom). The cathode and anode were connected to a 2000 Ω resistor using titanium wire. The water level was kept constant by daily replenishment.

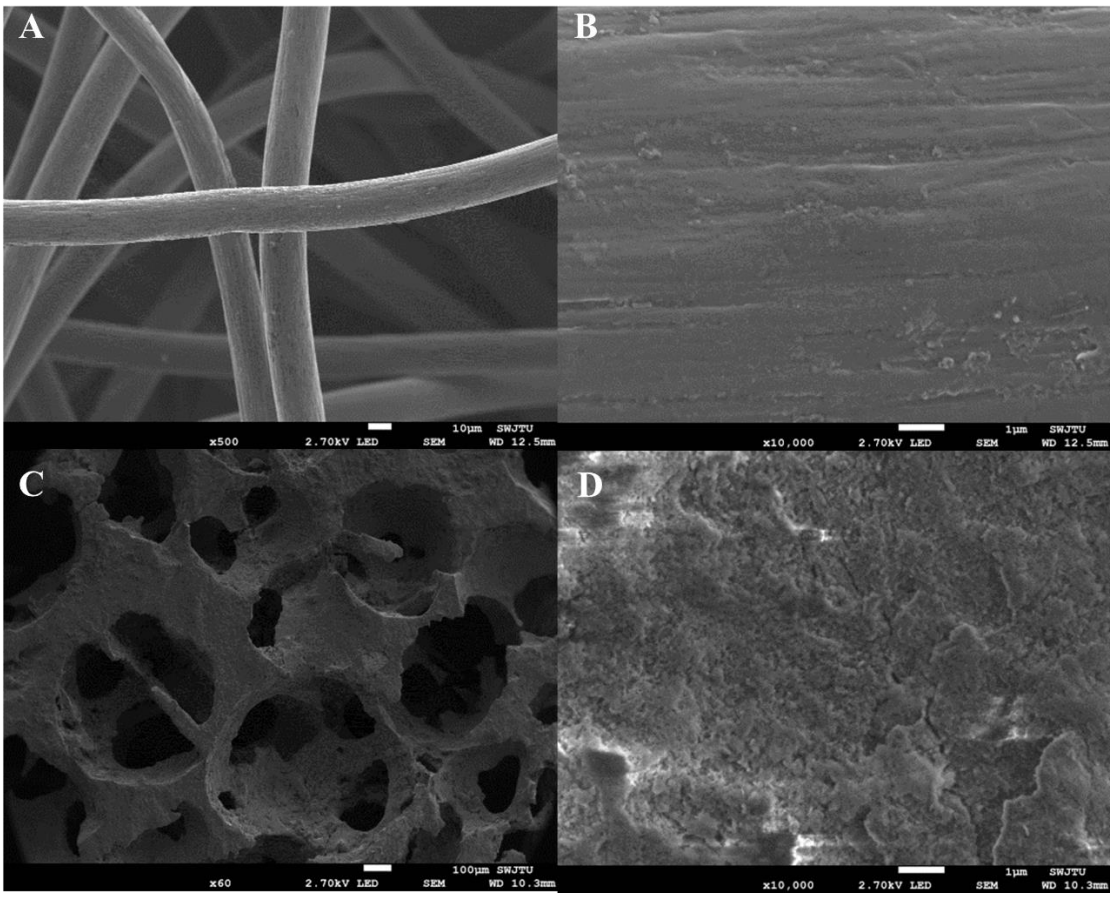


Fig. S2 Electrode material characterization. (A, B) SEM images of GF without catalyst loading; (C, D) SEM images of aluminum foam.

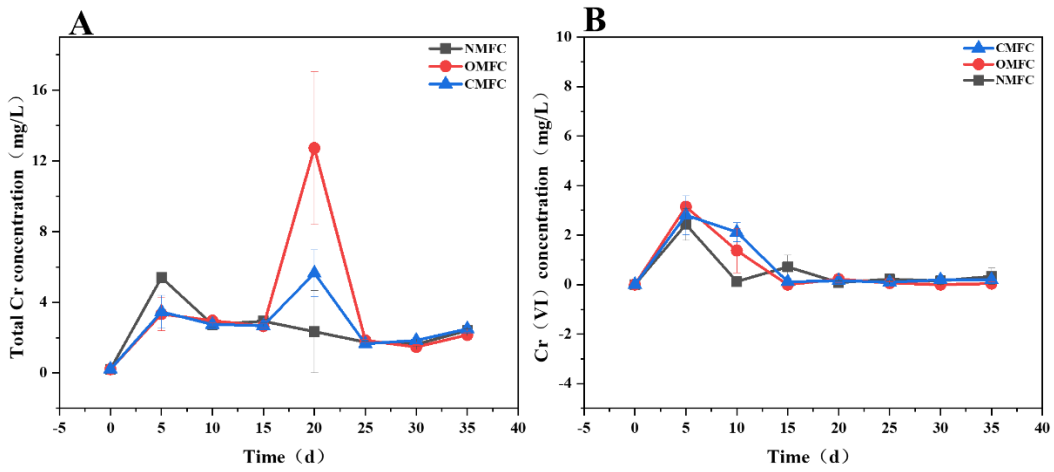


Fig. S3 Variation of (A) total chromium and (B) Cr(VI) in overlying water during SMFC operation.

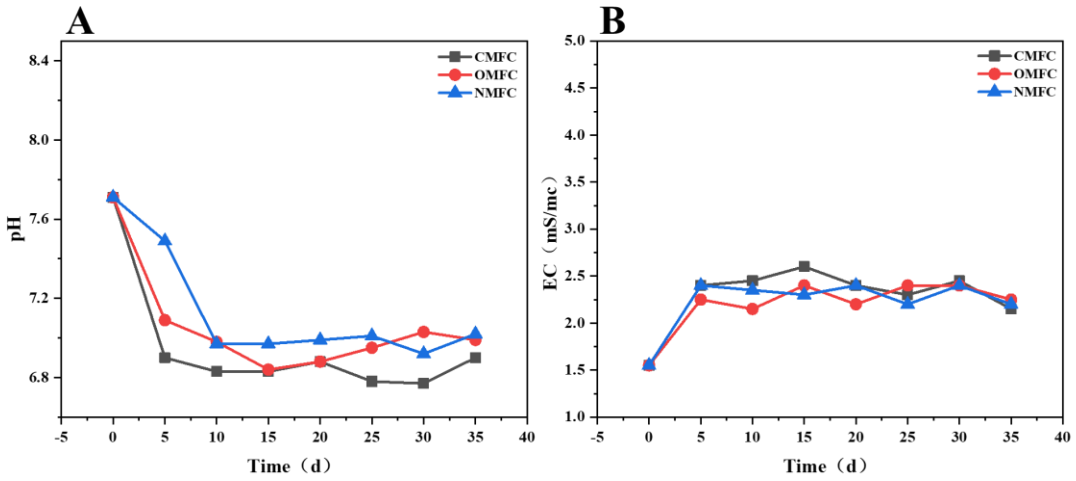


Fig. S4 pH (A), EC (B) variation curves of soil.

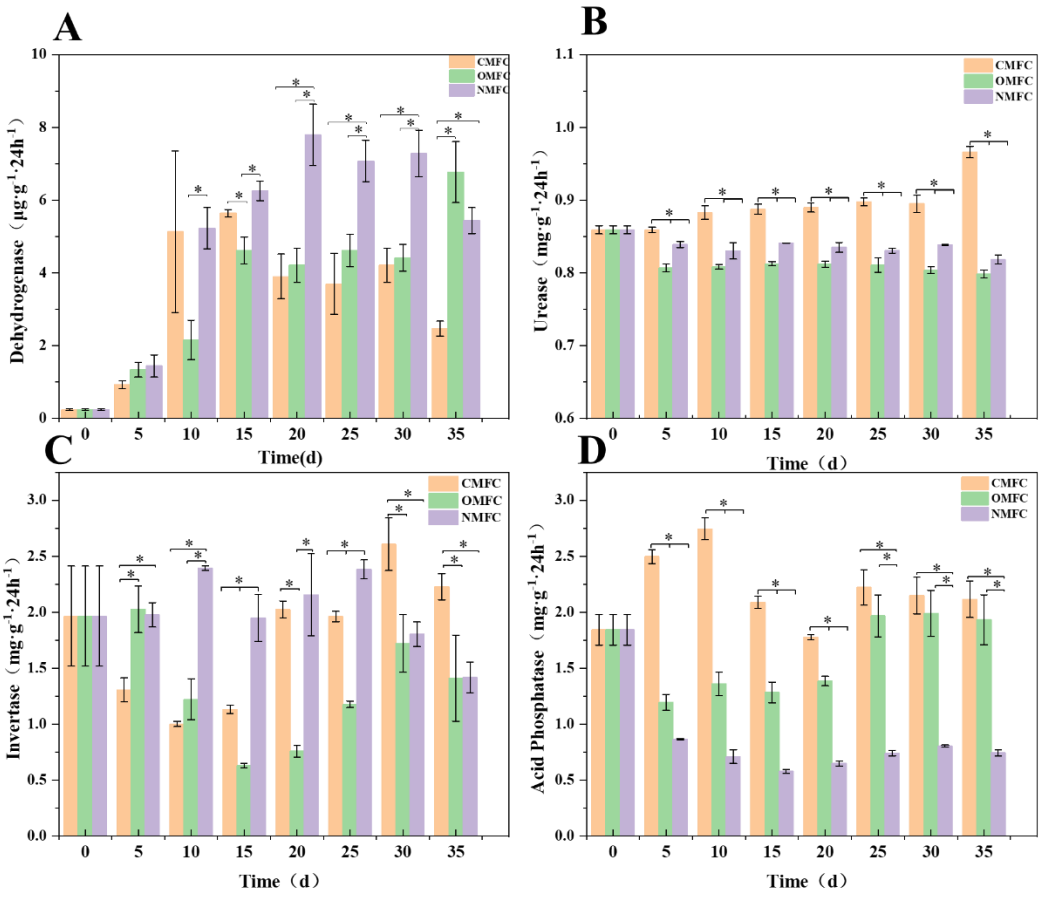


Fig. S5 Changes in soil enzyme activities during SMFC operation (A) Dehydrogenase (B) Urease (C) Invertase(D) Acid Phosphatase.

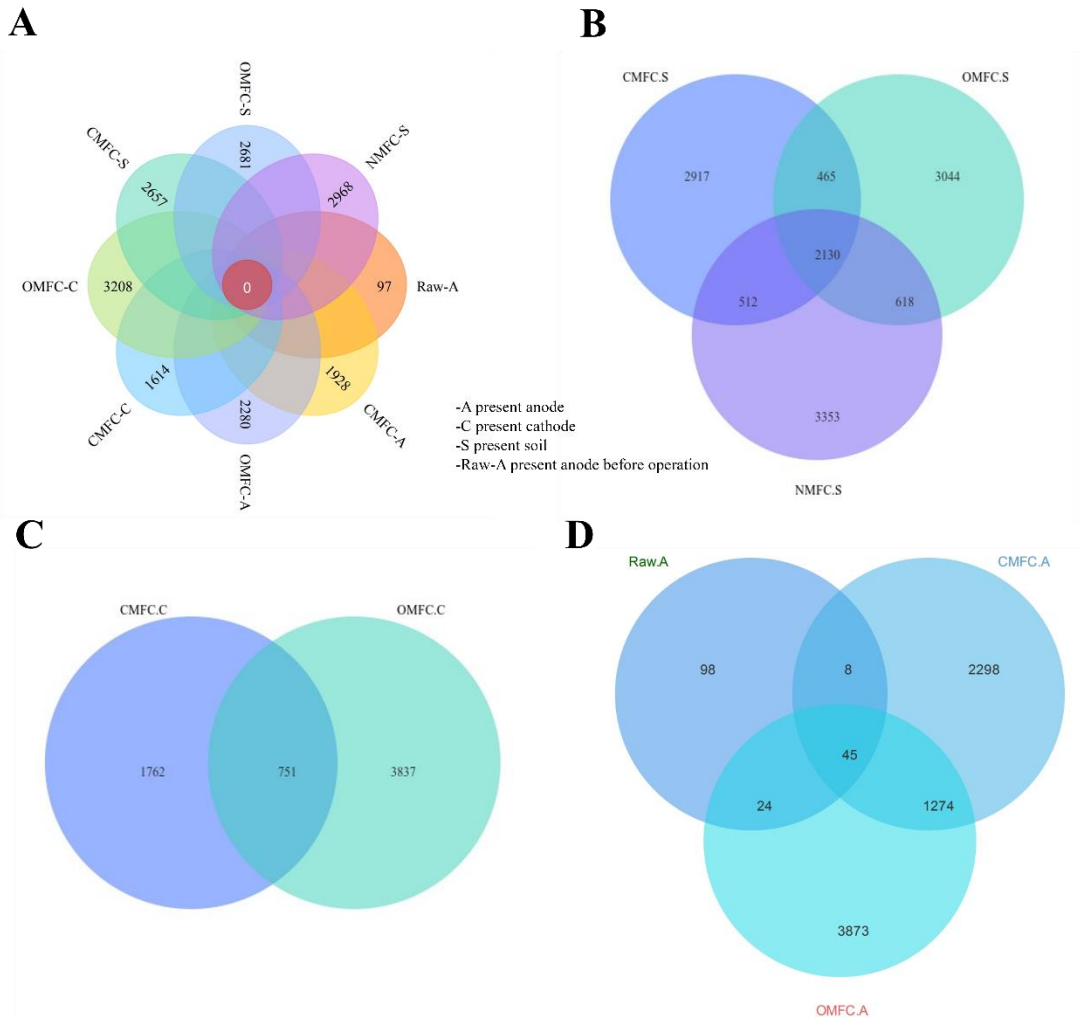
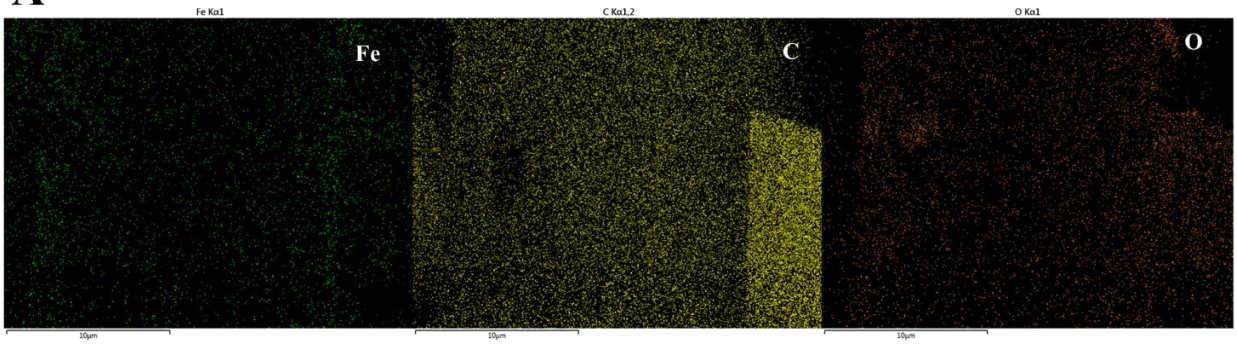
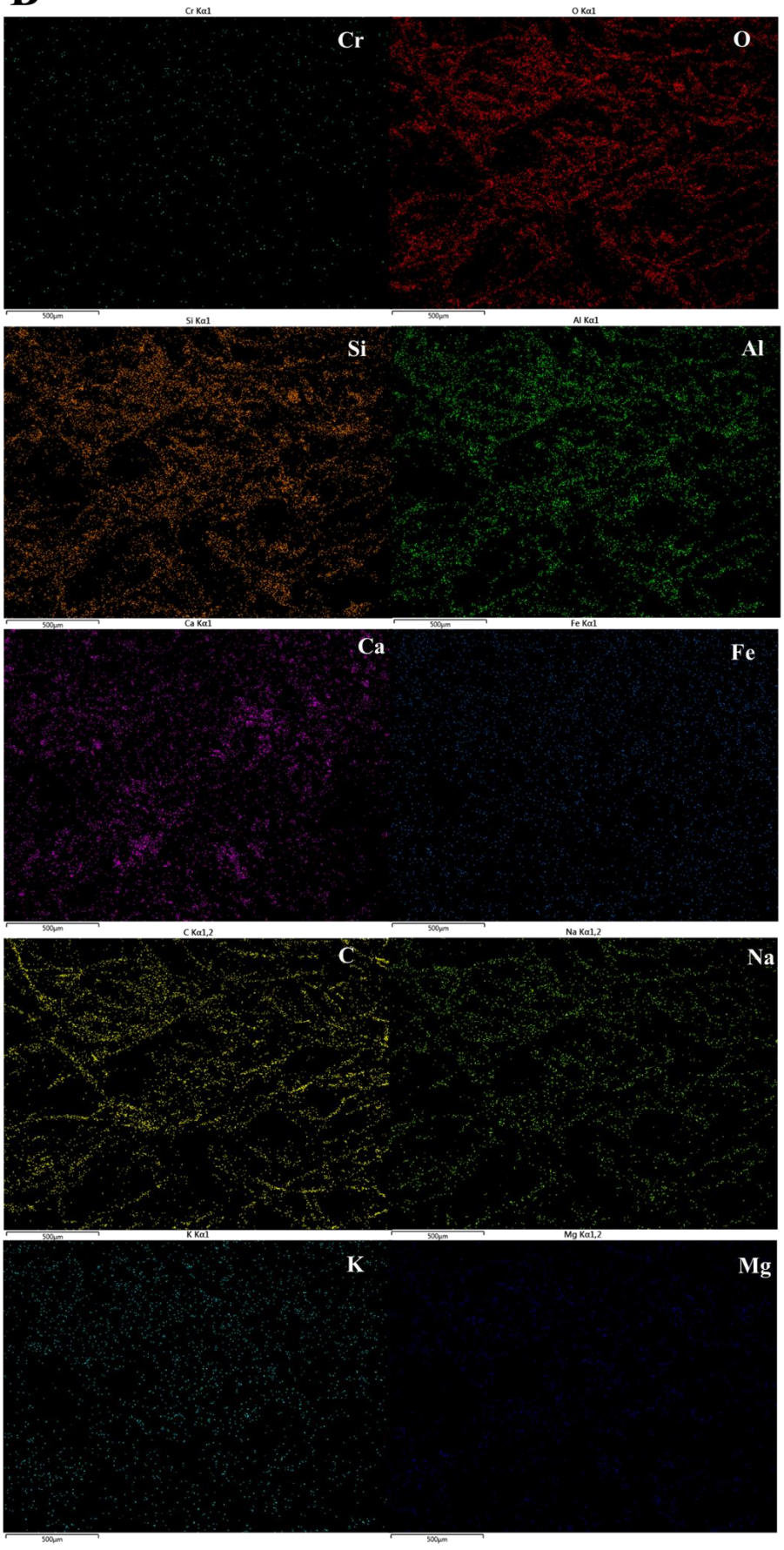


Fig. S6 Venn diagram on OTU level in different treatments

A



B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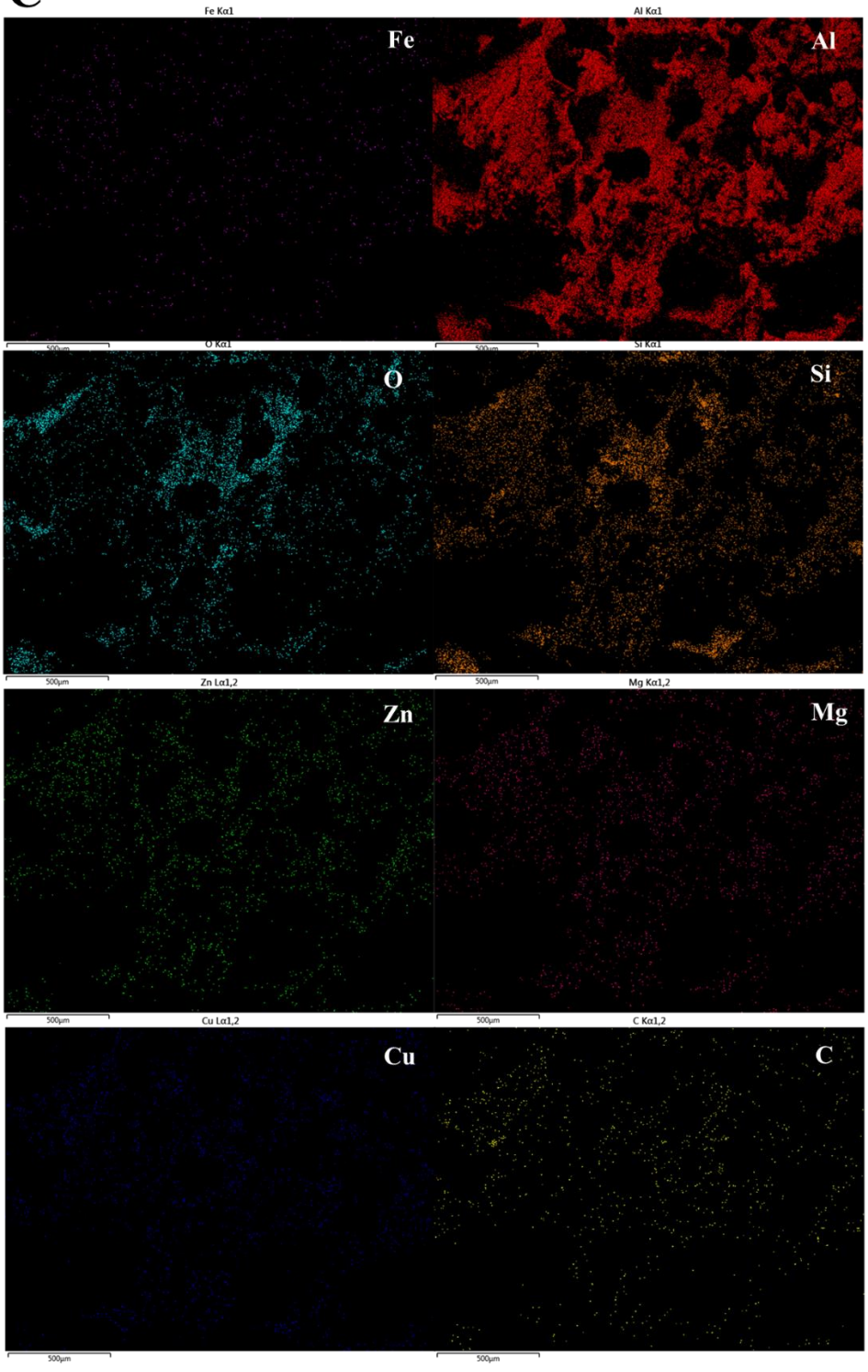
Cr
O Kα1
O
500μm
500μm
Si Kα1
Si
Al Kα1
Al
500μm
500μm
Ca Kα1
Ca
Fe Kα1
Fe
500μm
500μm
C Kα1,2
C
Na Kα1,2
Na
500μm
500μm
K Kα1
K
Mg Kα1,2
Mg
500μm
500μm

C


Fe Kα1
Fe
Al Kα1
Al
500μm
500μm
O Kα1
O
Si Kα1
Si
500μm
500μm
Zn Lα1,2
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Mg
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500μm
Cu Lα1,2
Cu
C Kα1,2
C
500μm
500μm

D

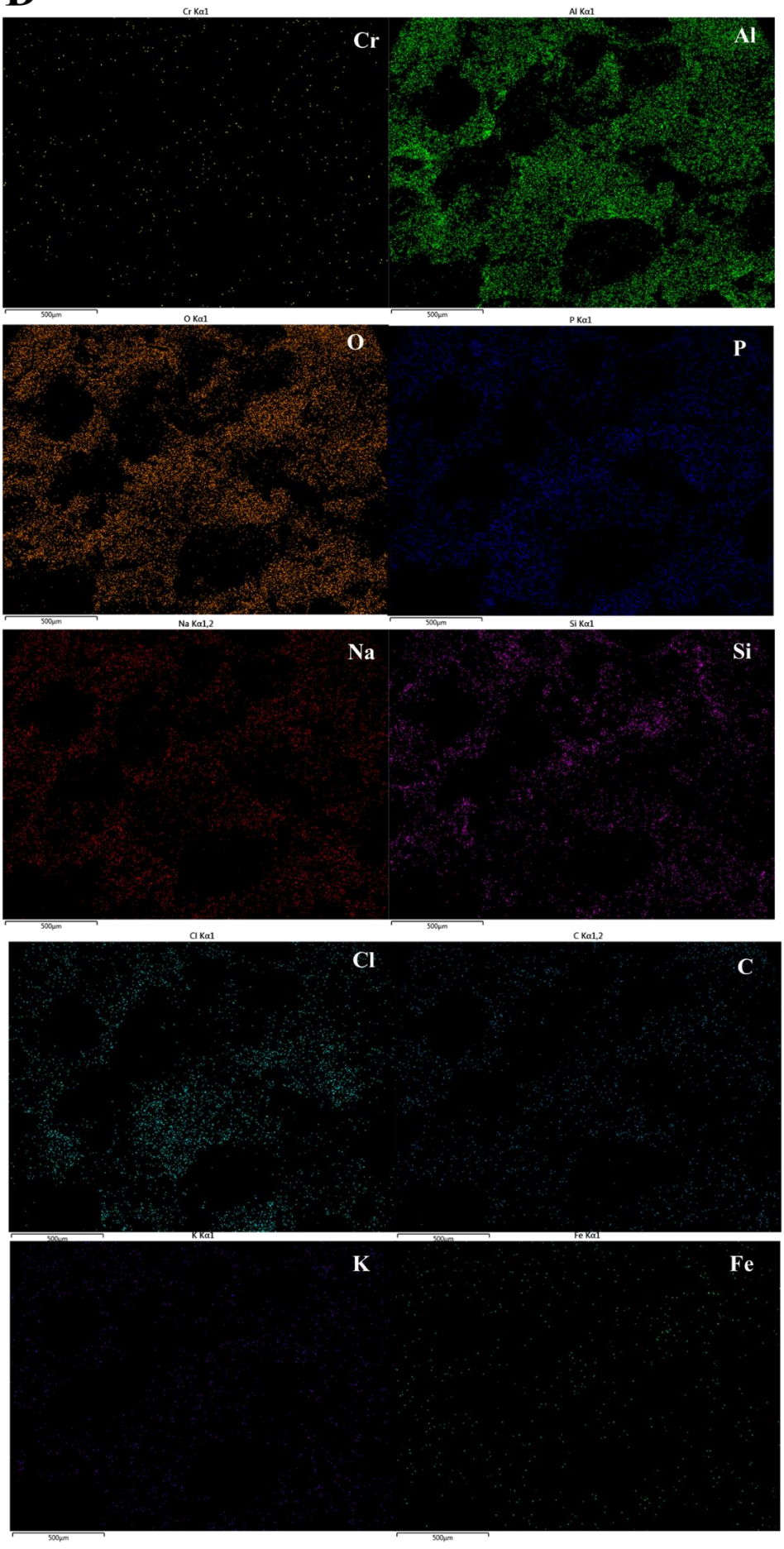
Cr Kα1
Cr
500μm
Al Kα1
Al
500μm
O Kα1
O
500μm
P Kα1
P
500μm
Na Kα1,2
Na
500μm
Si Kα1
Si
500μm
Cl Kα1
Cl
500μm
C Kα1,2
C
500μm
K Kα1
K
500μm
Fe Kα1
Fe
500μm

Fig. S7 Characterization of electrode materials before and after operation by EDS mapping. (A) EDS image of cathode loaded with Fe_3O_4; (B) EDS image of cathode after the SMFC operation; (C) EDS image of anode microorganisms; (D) EDS image of the anode after SMFC operation.

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