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# Fate of sulfamethoxazole and its corresponding resistance genes in a continuous flow biofilm electrode reactor -microbial fuel cell coupled constructed wetlands system

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# CONTENTS

# 1 Research background

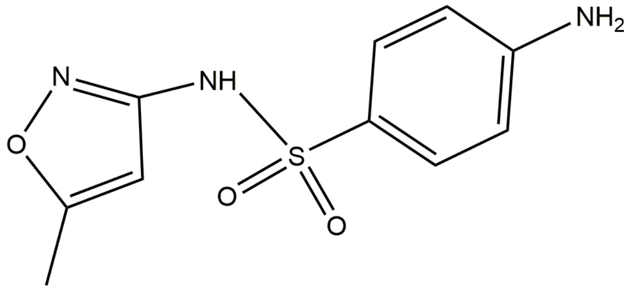
# Antibiotic

**Targeted pollutant :** Sulfamethoxazole ( SMX )

**Chemical equation :** $C_{10}H_{11}N_3O_3S$

**Log Kow :** 0.48

**Adsorption coefficient :** 0.6-31




**SMX**

Treatment of disease
Stimulation growth



Incomplete metabolism

Induces ARB&ARG

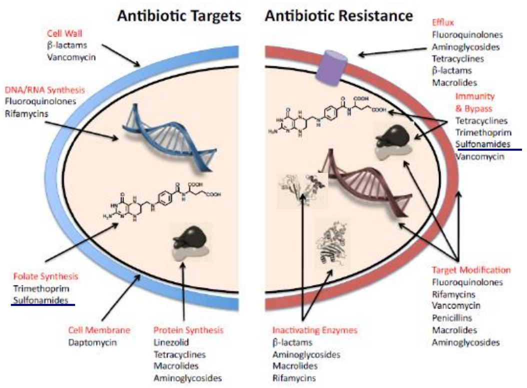

# Antibiotic resistance genes

**Target location：** Emerging pollutants

**Source：** Antibiotic induce, Replication error of gene

**Spread：** Vertical transfer; horizontal transfer ( including conjugative  transfer, natural transformation and transduction )

**Damage：** A serious threat to human health and safety



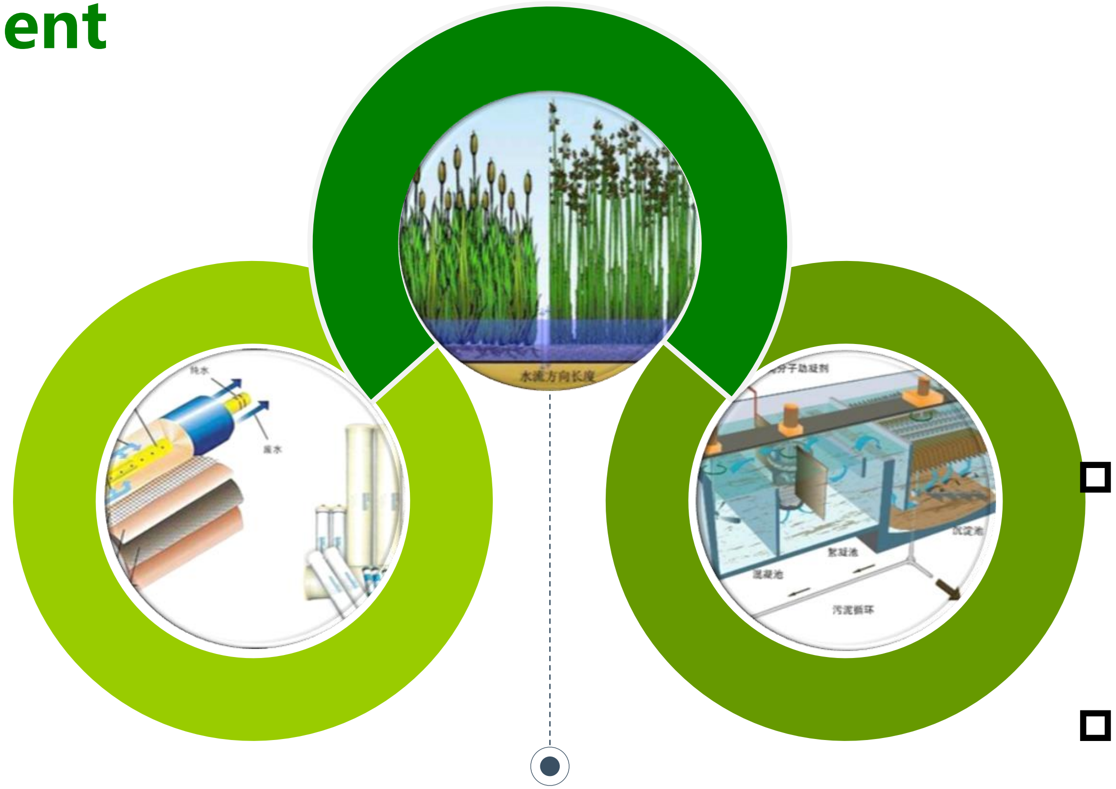

**Target resistance gene：** *sul I*、*sul II*、 *sul III*

Sewage treatment facilities

Sediment

Atmosphere

# Conventional treatment technique



## Physical method

- Antibiotic：Activate Carbon Adsorption Method、Sand-filtering and membrane filtration
- Resistance gene：sediment、filter and ultraviolet disinfection
- ✓ High-cost，Secondary pollutiom

## Biological method

- Antibiotic：Activated sludge technique、Constructed wetland
- Resistance gene：Activated sludge technique 、Anaerobic digestion、Constructed wetland
- ✓ Pollution-free，Low cost, High removal efficiency

## Chemical method

- chemical method：Coagulation precipitation method、Chemical oxidation technique
- Resistance gene：Lime stabilization method、Ozone and chlorine disinfection
- ✓ Intermediate products are poisonous，high costs
- ✓ Disinfection does not work for all ARGs

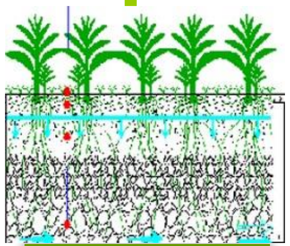
# Microbial fuel cell-constructed wetland coupled with biofilm-electrode reactors ( BER-MFC-CW )

In a CW-MFC system, a significant redox gradient can be developed along its height.

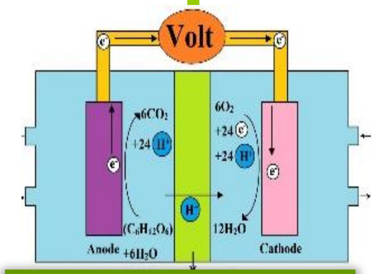
Aerobic environment could be regarded as cathode.

Filler absorption, Plant absorption, and Microbial degradation

**Improve the efficiency of antibiotic removal**



CW




MFC

**Reduce energy consumption**

Drives the biodegradation of biorefractory and toxic compound sand produce electricity

Anaerobic environment at the bottom could be regarded as an anode chamber 。

## Microbial fuel cell-constructed wetland (MFC-CW )

**electric energy**

**Antibiotic** → BER → MFC-CW →

**Biofilm-electrode reactors**

**Microbial fuel cell-constructed wetland**

- Need micro-current only
- Pretreatment antibiotics ( Biological and electrochemical action)

- MFC power generation capacity enhancement
- BER processing efficiency improved

## BER-MFC-CW

# 2 Research program

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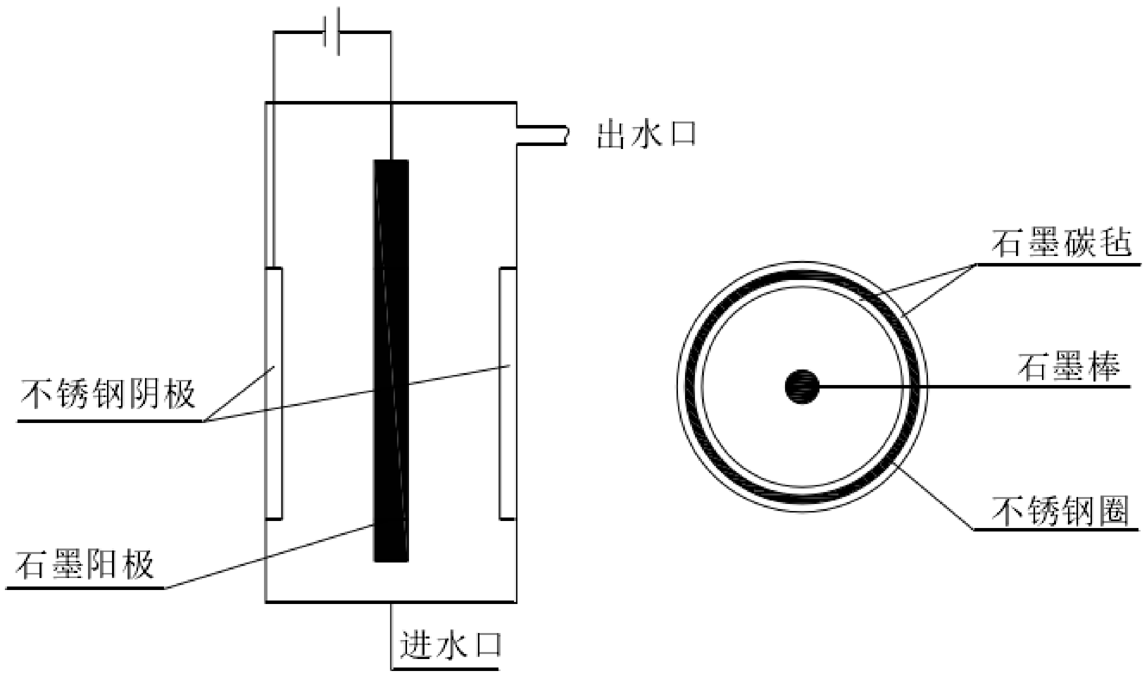
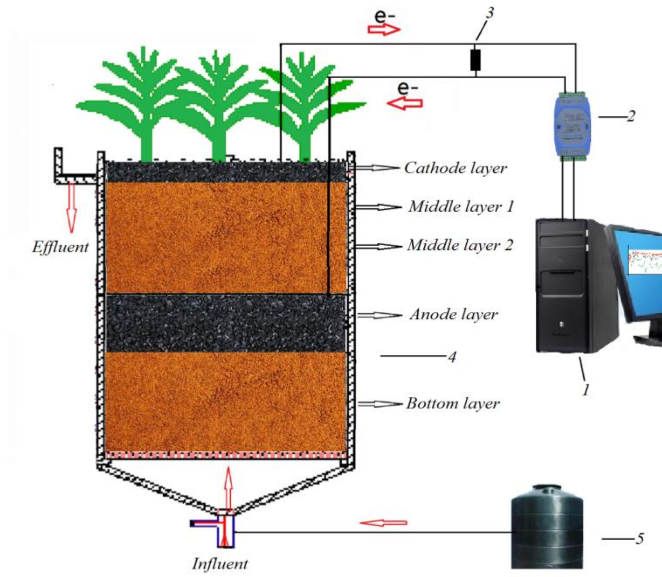
# Experiment device

## Microbial fuel cell-constructed wetland（ MFC-CW ）




- Plexiglass tub，diameter=15cm，Height=30cm。
- Up-flow
- Anode was graphite rod and cathode was stainless steel wire mesh

## Biofilm electrode reactor ( BER )




- Diameter=19cm，Height=32cm
- Up-flow
- Activated carbon and stainless steel wire mesh are used as electrode materials.
- The wetlands are filled with gravel and planted with celery

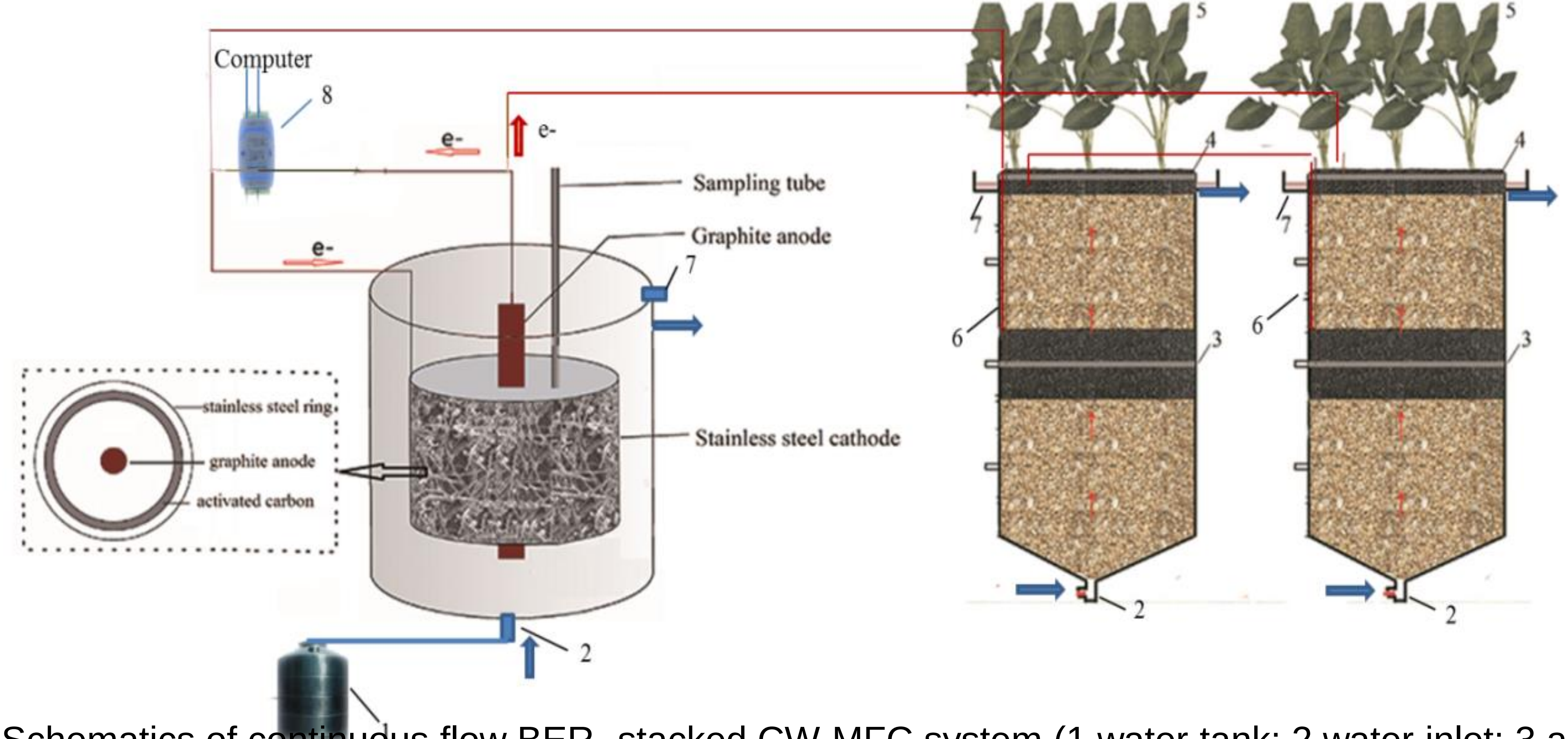
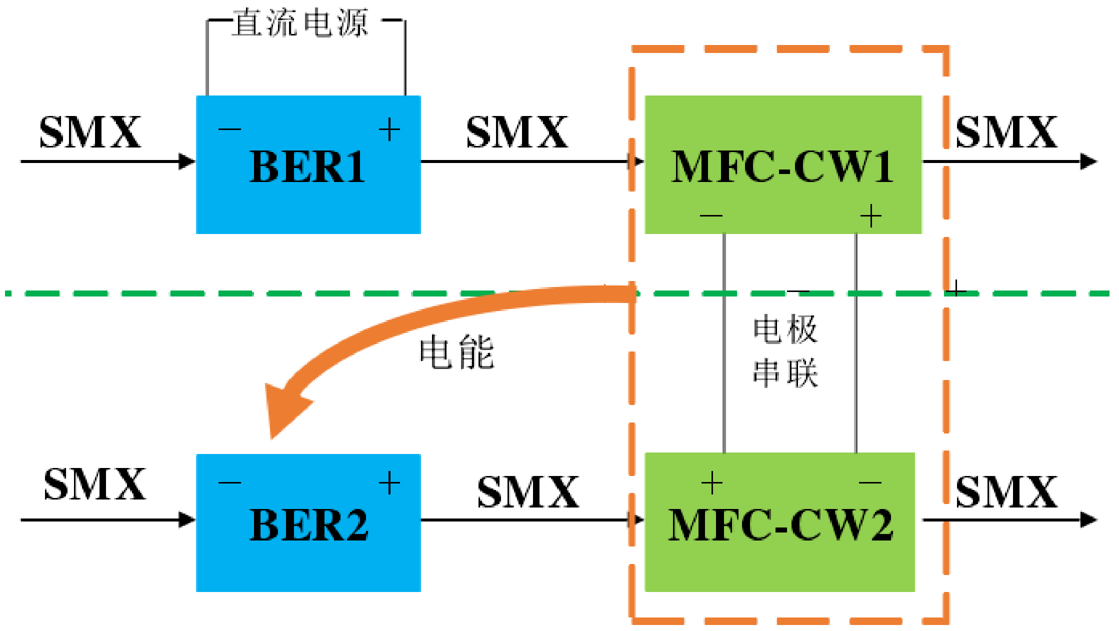



Fig. Schematics of continuous flow BER -stacked CW-MFC system (1 water tank; 2 water inlet; 3 anode of CW-MFC; 4 cathode of CW-MFC; 5 wetland plants; 6 middle layer; 7 water outlet; 8 data acquisition module)

# Phase 1

## BER-CW- MFCOperating parameters

| Reactor number | HRT | SMX（mg/L） | Power source |
|---|---|---|---|
| BER 1 | 2.5d | 2 | Direct current |
| BER 2 | 2.5d | 2 | powered by bioelectricity supplied by MFC-CW1, 2 in series |
| BER 3 | 2.5d | 4 | Direct current |
| BER 4 | 2.5d | 4 | powered by bioelectricity supplied by MFC-CW3, 4 in series |
| MFC-CW 1 | 2.5d | BER 1 effluent | In series |
| MFC-CW 2 | 2.5d | BER 2 effluent | |
| MFC-CW 3 | 2.5d | BER 3 effluent | In series |
| MFC-CW 4 | 2.5d | BER 4 effluent | |




MFC-CW 串联系统

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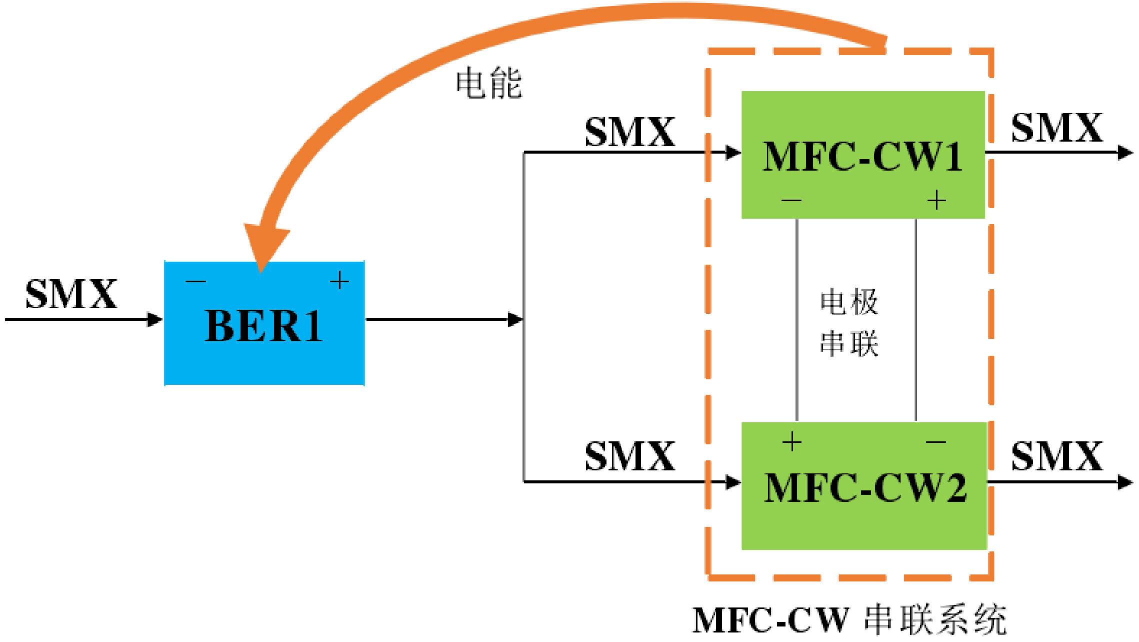
# Phase 2

## BER-MFC-CW Operating parameters

| Reactor number | HRT | SMX（mg/L） | Power source |
|---|---|---|---|
| BER 1 | 16h | 4 | powered by bioelectricity supplied by MFC-CW1, 2 in series |
| BER 2 | 8h | 4 | powered by bioelectricity supplied by MFC-CW3,4 in series |
| BER 3 | 4h | 4 | powered by bioelectricity supplied by MFC-CW5,6in series |
| MFC-CW 1<br>MFC-CW 2 | 32h | BER 1effluent | |
| MFC-CW 3<br>MFC-CW 4 | 16h | BER 2effluent | |
| MFC-CW 5<br>MFC-CW 6 | 8h | BER 3effluent | |



# 3 Research results and discussions

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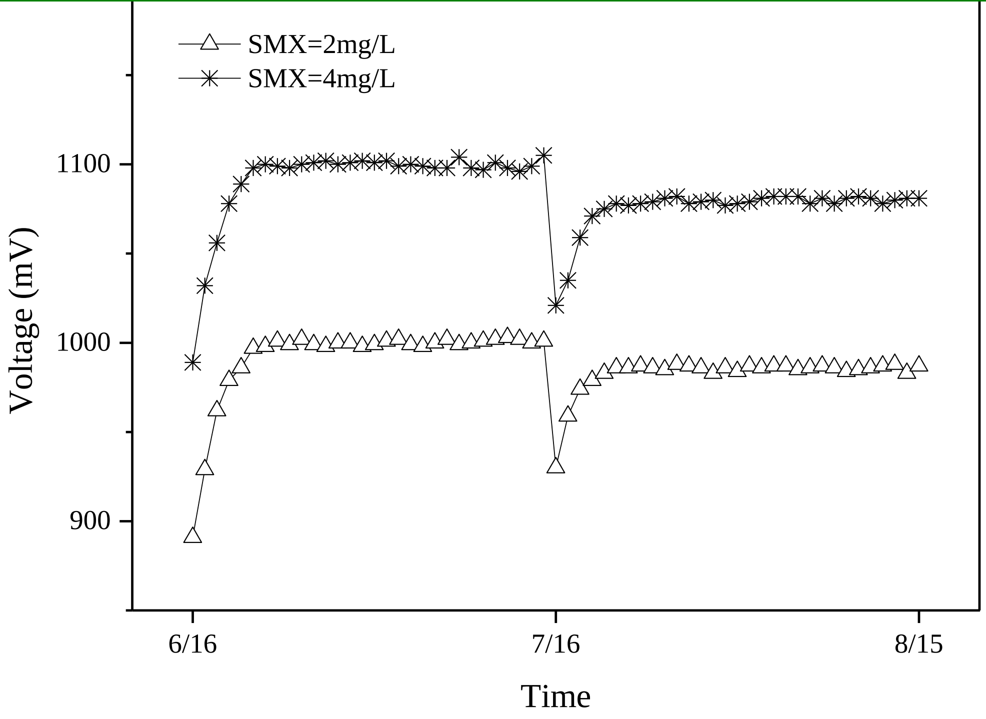
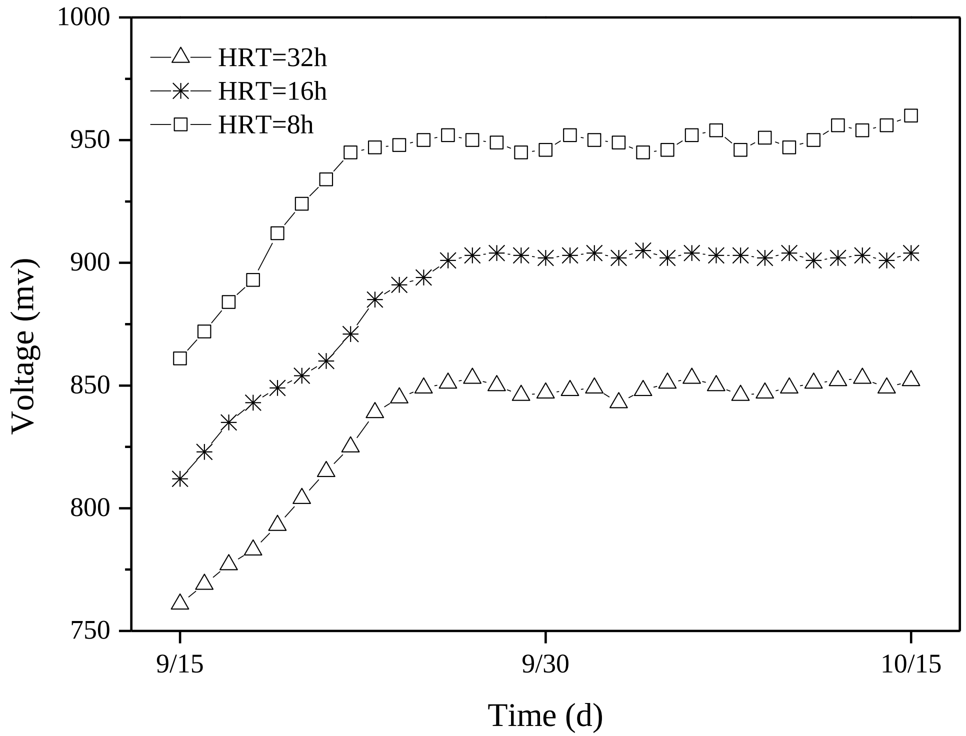
# BER-MFC-CW removal of antibiotics and simultaneous production of electricity

## Effect of SMX concentration on power generation in MFC-CW system




**T**he electrical performance of MFC-CW increased with the increase of SMX concentration and decreased with longer running time

## The influence of HRT on the power generation of MFC-CW system




The power performance of MFC-CW increases with the decrease of HRT

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# BER-MFC-CW removal of antibiotics and simultaneous production of electricity

## SMX concentration of BER-MFC-CW under different SMX concentrations

| 7.15（30d） | | | | | |
|---|---|---|---|---|---|
| | | Influent SMX (μg/L) | Effluent SMX (μg/L) | Removal efficiency （%） | Total removal efficiency （%） |
| BER-MFC-CW 1 (General composite system) | BER1<br>MFC-CW1 | 2000<br>285.4±23.1 | 285.4±23.1<br>14.2±2.8 | 85.73±1.16<br>95.02±0.98 | 99.29±0.14 |
| BER-MFC-CW 2 (Coupled system of matter and energy) | BER2<br>MFC-CW2 | 2000<br>207.9±21.7 | 207.9±21.7<br>12.1±2.1 | 89.61±1.09<br>94.10±1.01 | 99.39±0.11 |
| BER-MFC-CW 3 (General composite system) | BER3<br>MFC-CW3 | 4000<br>423.3±39.4 | 423.3±39.4<br>23.1±5.3 | 89.42±0.99<br>94.54±1.25 | 99.42±0.13 |
| BER-MFC-CW 4 (Coupled system of matter and energy | BER4<br>MFC-CW4 | 4000<br>382.4±37.5 | 382.4±37.5<br>25.8±4.9 | 90.44±0.94<br>93.25±1.28 | 99.35±0.12 |

- The total removal rate of SMX was as high as 99%
- BER removal rate of SMX was up to 90%.
- MFC-CW removal rate of SMX was up to 95%。
- The removal effect of SMX on the coupling system is slightly higher than that of the composite system

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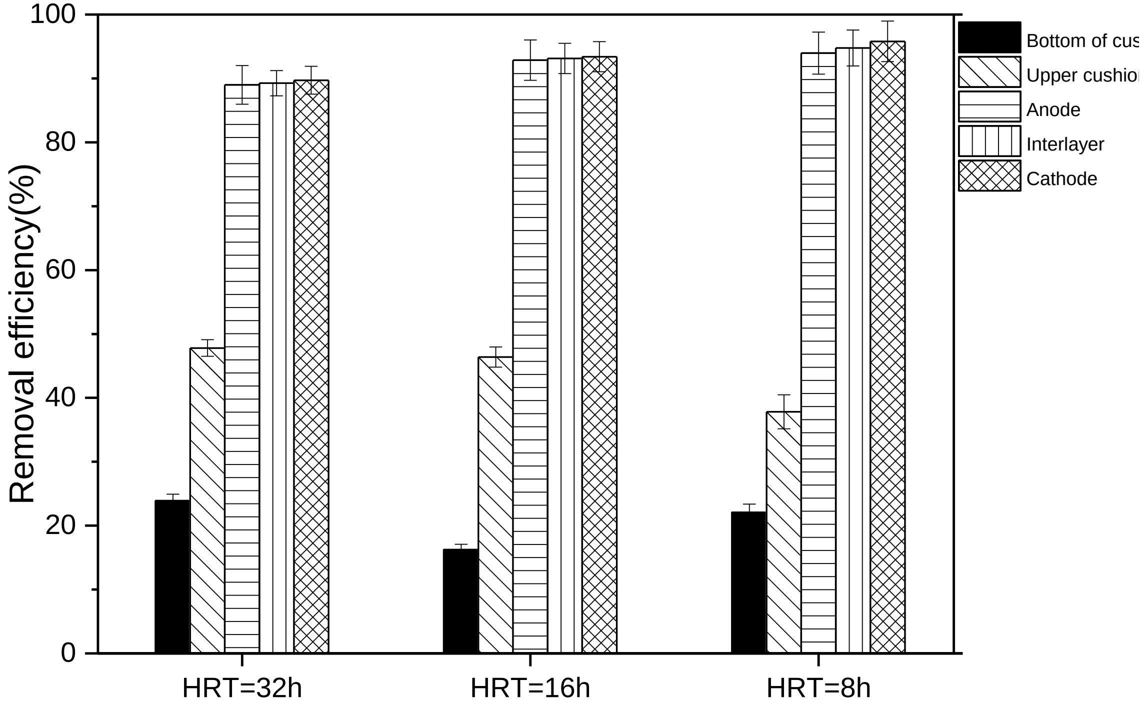
# BER-MFC-CW removal of antibiotics and simultaneous production of electricity

## SMX concentration of BER-MFC-CW under different HRT

| Oct 15th | | | | | | |
|---|---|---|---|---|---|---|
| | | HRT | Influent SMX (μg/L) | Effluent SMX (μg/L) | Unit removal rate （%） | Total removal rate （%） |
| **BER-MFC-CW 1 Coupled system of matter and energy** | BER1<br>MFC-CW1, 2 | 16h<br>32h | 4000<br>954.3±73.8 | 954.3±73.8<br>90.3±13.1 | 75.00±1.84<br>90.54±1.37 | 97.74±0.33 |
| **BER-MFC-CW 2 Coupled system of matter and energy** | BER2<br>MFC-CW3, 4 | 8h<br>16h | 4000<br>1507.7±123.1 | 1507.7±123.1<br>94.7±13.9 | 62.50±3.08<br>93.72±0.92 | 97.63±0.35 |
| **BER-MFC-CW 3 Coupled system of matter and energy** | BER3<br>MFC-CW5, 6 | 4h<br>8h | 4000<br>2140.5±243.5 | 2140.5±243.5<br>98.2±15.2 | 48.00±6.09<br>95.33±0.71 | 97.54±0.38 |

- **1The total removal rate of SMX in BER-MFC-CW system is as high as 97%.2With the decrease of HRT, the removal rate of SMX decreased slightly. The removal rate of SMX by BER decreased with HRT.**

## The removal rate of antibiotics in MFC-CW in different layer




- Antibiotics are mainly removed in the anodic layer. Removal efficiency was about 42-55%.

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# Characteristics of resistant genes in BER-MFC-CW effluent

## Absolute abundance：Logarithm of absolute copy number of resistant genes in 1ml water

### Effect of SMX concentration on absolute abundance of resistant genes

| | | 30d *sulI* | 60d *sulI* | 30d *sulII* | 60d *sulII* | 30d *sulIII* | 60d *sulIII* | 30d 16S | 60d 16S |
|---|---|---|---|---|---|---|---|---|---|
| 2mg/L | BER1 | 6.26 | 5.71 | 6.16 | 5.59 | 3.62 | 3.06 | 7.63 | 7.07 |
| 2mg/L | BER2 | 6.02 | 5.76 | 5.91 | 5.61 | 3.41 | 3.14 | 7.52 | 7.24 |
| 4mg/L | BER3 | 6.87 | 6.64 | 6.79 | 6.55 | 4.24 | 4.00 | 7.99 | 7.75 |
| 4mg/L | BER4 | 6.60 | 6.41 | 6.51 | 6.29 | 3.94 | 3.75 | 7.78 | 7.58 |
| | MFC-CW1 | 5.02 | 5.13 | 4.92 | 5.02 | 2.35 | 2.46 | 6.62 | 6.75 |
| | MFC-CW2 | 4.82 | 4.88 | 4.72 | 4.76 | 2.12 | 2.20 | 6.53 | 6.59 |
| | MFC-CW3 | 5.70 | 5.64 | 5.64 | 5.59 | 3.05 | 2.98 | 7.13 | 7.07 |
| | MFC-CW4 | 5.52 | 5.47 | 5.46 | 5.43 | 2.89 | 2.85 | 7.02 | 6.97 |

Maximum value

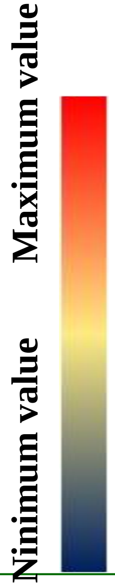
Minimum value

- ✓ ***sulI>sulII>sulIII;* BER>CW-MFC**
- ✓ **4mg/L was 0,6-0.8 orders of magnitude higher than 2mg/L group.**
- ✓ ***The absolute abundance of <u>sul</u> genes and bacteria decreases with time***
- ✓ **Influent（no *sul* gene）—the effluent of BER（produce *sul* gene）—effluent of MFC-CW（ *Sulfonamide gene reduction*）**
- ✓ **MFC-CW had a 1-1.3 order of magnitude removal of the two Sul genes and a 0.76-1.01 order of magnitude removal for bacteria at 30 d.**

# Characteristics of resistant genes in BER-MFC-CW effluent

**The influence of HRT on absolute abundance of resistance genes**

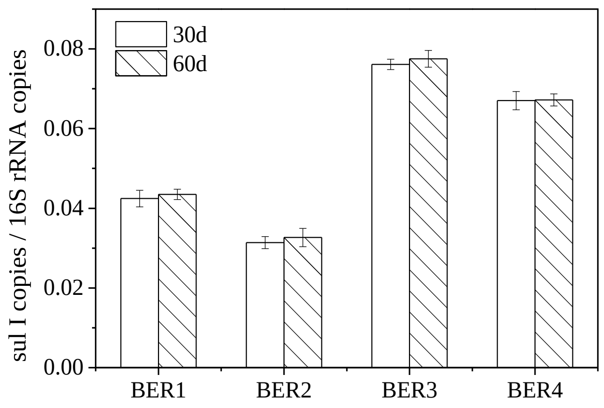
| | | *sulI* | *sulII* | *sulIII* | 16S |
|---|---|---|---|---|---|
| HRT=16h | BER1 | 5.97 | 5.85 | 3.31 | 7.05 |
| HRT=8h | BER2 | 6.17 | 6.03 | 3.71 | 7.12 |
| HRT=4h | BER3 | 6.34 | 6.21 | 3.92 | 7.21 |
| | MFC-CW1, 2 | 5.18 | 5.03 | 2.49 | 6.53 |
| | MFC-CW3, 4 | 5.40 | 5.26 | 2.73 | 6.64 |
| | MFC-CW5, 6 | 5.83 | 5.70 | 3.10 | 6.97 |



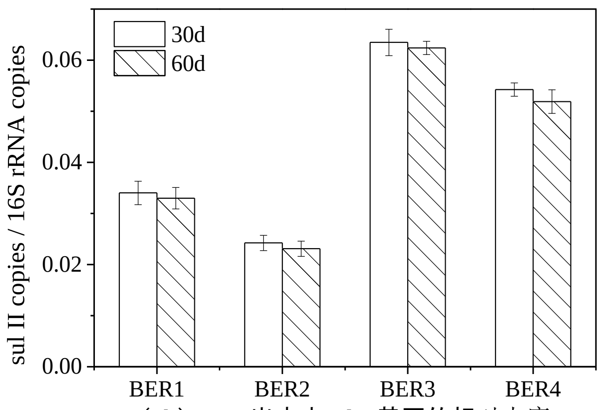

- **The absolute abundance of *sul* gene in BER and MFC-CW effluent increased with the decrease of HRT**
- **MFC-CW had a 0.5-1 order of magnitude removal of the three *sul* genes and a 0.25-0.54 order of magnitude removal for bacteria**
- **With the decrease of HRT, the removal ability of MFC-CW to *sul* gene also decreased**

# Characteristics of resistant genes in BER-MFC-CW effluent

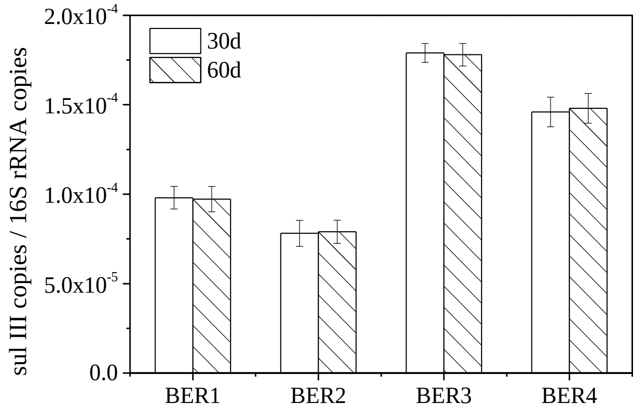
## Effect of SMX concentration on relative abundance of resistant genes



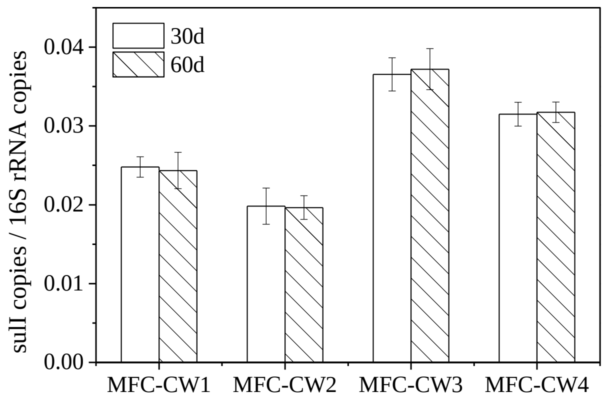

（a）BER出水中sul I基因的相对丰度



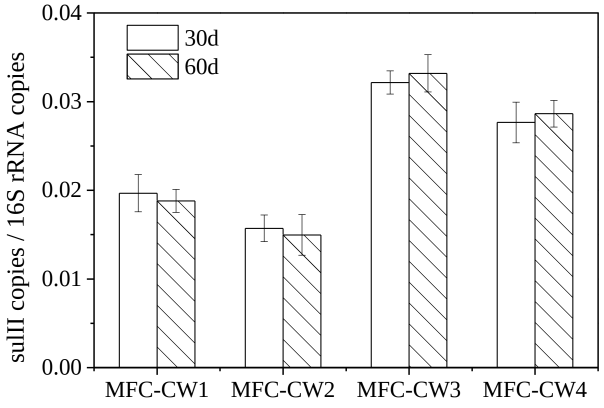

（b）BER出水中sul II基因的相对丰度



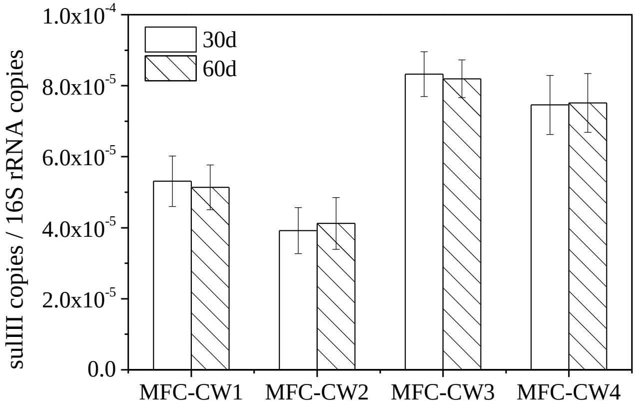

（c）BER出水中sul III基因的相对丰度




（a）MFC-CW出水中sul I基因的相对丰度




（b）MFC-CW出水中sul II基因的相对丰度

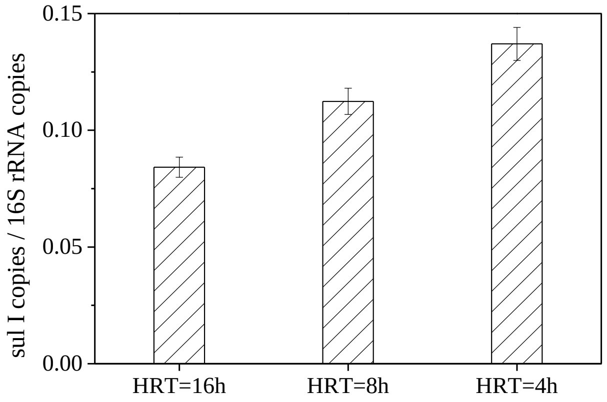



（c）MFC-CW出水中sul III基因的相对丰度

- ***sulI>sulII>sulIII***
- **Composite system > Coupled system**
- **BER>MFC-CW**
- **BER：sul（2mg/L）=1/2 sul（4mg/L）**
- **MFC-CW：sul（2mg/L）=2/3 sul（4mg/L）**

# Characteristics of resistant genes in BER-MFC-CW effluent

## The influence of HRT on relative abundance of resistance genes



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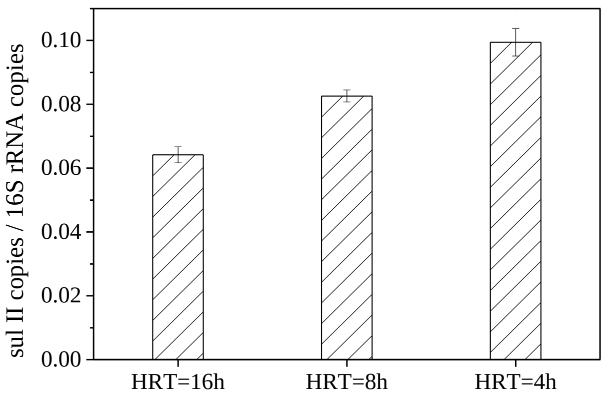
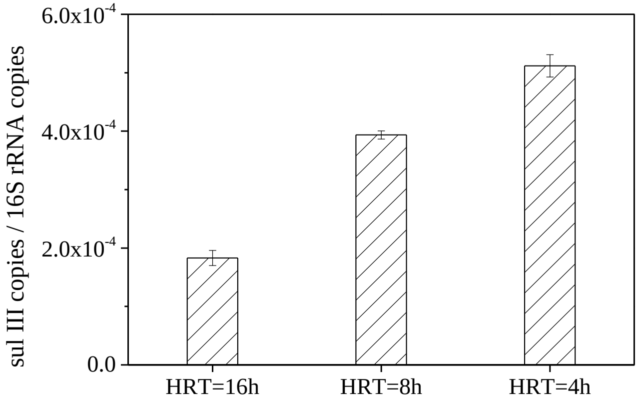
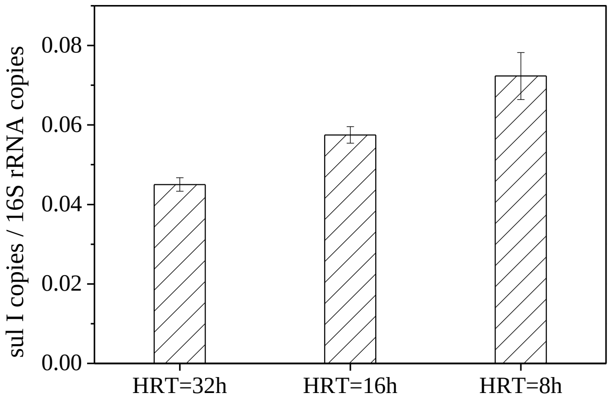


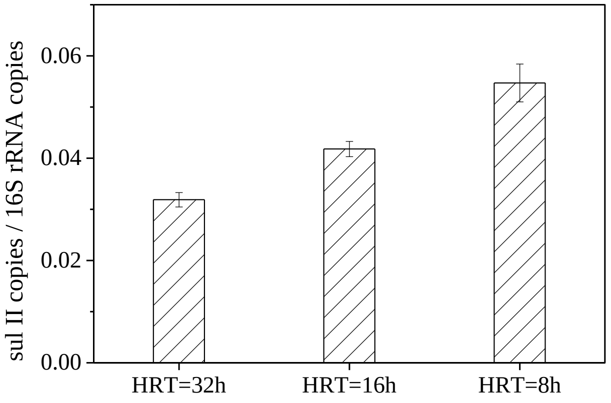
（b）BER出水中sul II基因的相对丰度



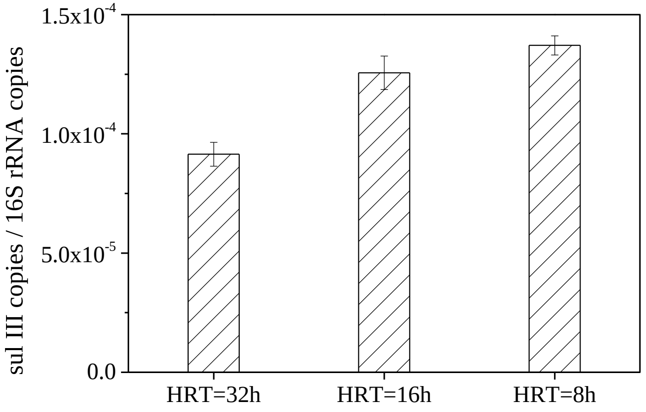
（c）BER出水中sul III基因的相对丰度



（a）MFC-CW出水中sul I基因的相对丰度



（b）MFC-CW出水中sul II基因的相对丰度



（c）MFC-CW出水中sul III基因的相对丰度

- ***sulI>sulII>sulIII***
- **BER>MFC-CW**
- **The relative abundance of *sul* resistance gene increases with the decrease of HRT**

# THANKS