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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1 Research background
**Antibiotic**

**Targeted pollutant**: Sulfamethoxazole (SMX)

**Chemical equation**: \( C_{10}H_{11}N_{3}O_{3}S \)

**Log Kow**: 0.48

**Adsorption coefficient**: 0.6-31

- **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

Conventional treatment technique

Physical method

- Resistance gene: sediment, filter and ultraviolet disinfection
- High-cost, Secondary pollution

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

Biological method

- Antibiotic: Activated sludge technique, Constructed wetland
- Resistance gene: Activated sludge technique, Anaerobic digestion, Constructed wetland
- Pollution-free, Low cost, High removal efficiency
Microbial fuel cell-constructed wetland coupled with biofilm-electrode reactors (BER-MFC-CW)

Aerobic environment could be regarded as cathode.

Filler absorption, Plant absorption, and Microbial degradation Improve the efficiency of antibiotic removal

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.

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

Antibiotic

BER

Biofilm-electrode reactors

MFC-CW

Need micro-current only

Pretreatment antibiotics (Biological and electrochemical action)

MFC power generation capacity enhancement

BER processing efficiency improved
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- MFC Operating parameters

<table>
<thead>
<tr>
<th>Reactor number</th>
<th>HRT</th>
<th>SMX (mg/L)</th>
<th>Power source</th>
</tr>
</thead>
<tbody>
<tr>
<td>BER 1</td>
<td>2.5d</td>
<td>2</td>
<td>Direct current</td>
</tr>
<tr>
<td>BER 2</td>
<td>2.5d</td>
<td>2</td>
<td>powered by bioelectricity supplied by MFC-CW1, 2 in series</td>
</tr>
<tr>
<td>BER 3</td>
<td>2.5d</td>
<td>4</td>
<td>Direct current</td>
</tr>
<tr>
<td>BER 4</td>
<td>2.5d</td>
<td>4</td>
<td>powered by bioelectricity supplied by MFC-CW3, 4 in series</td>
</tr>
<tr>
<td>MFC-CW 1</td>
<td>2.5d</td>
<td>BER 1 effluent</td>
<td>In series</td>
</tr>
<tr>
<td>MFC-CW 2</td>
<td>2.5d</td>
<td>BER 2 effluent</td>
<td>In series</td>
</tr>
<tr>
<td>MFC-CW 3</td>
<td>2.5d</td>
<td>BER 3 effluent</td>
<td>In series</td>
</tr>
<tr>
<td>MFC-CW 4</td>
<td>2.5d</td>
<td>BER 4 effluent</td>
<td>In series</td>
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</tbody>
</table>
## Phase 2

### BER-MFC-CW Operating parameters

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

Diagram: 
- SMX enters the system and is treated in BER1 and MFC-CW1.
- The treated SMX is then directed to MFC-CW2 for additional treatment.
- The final effluent is collected from MFC-CW2.

Legend: 
- SMX: Substrate Moved eXchange
- MFC-CW: Bioelectrochemical Reactor and Constructed Wetland System
- BER: Bioreactor
3 Research results and discussions
BER-MFC-CW removal of antibiotics and simultaneous production of electricity

The electrical performance of MFC-CW increased with the increase of SMX concentration and decreased with longer running time.

The power performance of MFC-CW increases with the decrease of HRT.
BER-MFC-CW removal of antibiotics and simultaneous production of electricity

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

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

- 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.
**BER-MFC-CW removal of antibiotics and simultaneous production of electricity**

SMX concentration of BER-MFC-CW under different HRT

<table>
<thead>
<tr>
<th>Oct 15th</th>
<th></th>
<th>Influent SMX (μg/L)</th>
<th>Effluent SMX (μg/L)</th>
<th>Unit removal rate (%)</th>
<th>Total removal rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BER-MFC-CW 1 Coupled system of matter and energy</td>
<td>BER1 MFC-CW1, 2</td>
<td>16h</td>
<td>4000</td>
<td>954.3 ± 73.8</td>
<td>954.3 ± 73.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32h</td>
<td>954.3 ± 73.8</td>
<td>90.3 ± 13.1</td>
<td></td>
</tr>
<tr>
<td>BER-MFC-CW 2 Coupled system of matter and energy</td>
<td>BER2 MFC-CW3, 4</td>
<td>8h</td>
<td>4000</td>
<td>1507.7 ± 123.1</td>
<td>1507.7 ± 123.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16h</td>
<td>1507.7 ± 123.1</td>
<td>94.7 ± 13.9</td>
<td></td>
</tr>
<tr>
<td>BER-MFC-CW 3 Coupled system of matter and energy</td>
<td>BER3 MFC-CW5, 6</td>
<td>4h</td>
<td>4000</td>
<td>2140.5 ± 243.5</td>
<td>2140.5 ± 243.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8h</td>
<td>2140.5 ± 243.5</td>
<td>98.2 ± 15.2</td>
<td></td>
</tr>
</tbody>
</table>

1. The total removal rate of SMX in BER-MFC-CW system is as high as 97%.
2. With the decrease of HRT, the removal rate of SMX decreased slightly. The removal rate of SMX by BER decreased with HRT.
Antibiotics are mainly removed in the anodic layer. Removal efficiency was about 42-55%.

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

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

√ 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 sul 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

- 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

- $sulI > sulII > sulIII$
- 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

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