

**Removal of artificial sweeteners and their effects on microbial  
communities in sequencing batch reactors**

Shaoli Li, Jinju Geng\*, Gang Wu, Xingsheng Gao,  
Yingying Fu, Hongqiang Ren

State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment,  
Nanjing University, Nanjing 210023, P.R. China.

\*Corresponding author: Tel: +86 25 89680360. E-mail: jjgeng@nju.edu.cn

**Table S1. ASWs removals (%) in different samples during the sludge A and sludge B**

| Sludge   | Time(h) | B1            | B2          | B3          | B4          |
|----------|---------|---------------|-------------|-------------|-------------|
| Sludge A | 0.25    | 4.55 ± 0.32   | 1.26 ± 0.19 | 3.23 ± 0.20 | 0.03 ± 0.20 |
|          | 2       | 11.77 ± 1.12  | 2.58 ± 0.67 | 4.18 ± 0.16 | 1.23 ± 0.09 |
|          | 4       | 12.20 ± 0.34  | 4.96 ± 0.34 | 6.32 ± 1.12 | 1.85 ± 0.22 |
|          | 8       | 16.09 ± 2.56  | 4.23 ± 2.21 | 5.01 ± 0.23 | 0.60 ± 0.05 |
|          | 12      | 20.08 ± 3.59  | 5.27 ± 0.08 | 1.69 ± 0.89 | 0.75 ± 0.18 |
|          | 24      | 36.47 ± 4.28  | 3.65 ± 0.23 | 2.56 ± 0.32 | 0.28 ± 0.69 |
|          | 48      | 41.34 ± 1.02  | 5.59 ± 1.85 | 1.58 ± 0.15 | 0.03 ± 0.26 |
|          | 96      | 42.92 ± 0.39  | 7.91 ± 0.39 | 5.00 ± 2.30 | 3.08 ± 0.10 |
| Sludge B | 0.25    | 12.56 ± 0.59  | 2.46 ± 0.05 | 3.32 ± 0.32 | 0.98 ± 0.02 |
|          | 2       | 24.89 ± 1.89  | 4.25 ± 0.69 | 1.83 ± 0.04 | 3.21 ± 0.45 |
|          | 4       | 43.26 ± 0.37  | 2.15 ± 0.47 | 1.54 ± 1.16 | 5.17 ± 0.27 |
|          | 8       | 77.59 ± 5.23  | 4.39 ± 2.08 | 7.91 ± 0.76 | 0.55 ± 0.06 |
|          | 12      | 95.01 ± 4.16  | 6.12 ± 0.07 | 3.98 ± 0.78 | 0.51 ± 0.14 |
|          | 24      | 102.53 ± 2.90 | 9.45 ± 0.13 | 5.67 ± 0.47 | 0.05 ± 0.34 |
|          | 48      | 100.25 ± 1.02 | 6.31 ± 1.89 | 3.45 ± 0.15 | 0.09 ± 0.62 |
|          | 96      | 99.92 ± 2.03  | 6.09 ± 0.36 | 3.18 ± 1.32 | 1.18 ± 0.13 |

**Table S2. Physico-chemical properties of wastewater from sample sites**

| WWTP               | Process     | COD(mg L <sup>-1</sup> ) | NH <sub>4</sub> <sup>+</sup> -N(mg L <sup>-1</sup> ) | TN(mg L <sup>-1</sup> ) | pH  |
|--------------------|-------------|--------------------------|------------------------------------------------------|-------------------------|-----|
| Full-scale<br>WWTP | Influent    | 107                      | 19.9                                                 | 19.9                    | 7.7 |
|                    | Anaerobic   | 53                       | 6.7                                                  | 11.5                    | 7.1 |
|                    | Aerobic     | 31                       | 2                                                    | 7.2                     | 6.7 |
|                    | Coagulation | 17                       | 1.7                                                  | 6.9                     | 7.3 |
|                    | BAF         | 15                       | 1.8                                                  | 6.7                     | 7.4 |
|                    | UV          | 12                       | 1.2                                                  | 5.7                     | ND  |

ND: Not Detected

**Table S3. The outline of artificial sweeteners concentrations conditions in SBRs**

| Reactor code   | Artificial sweeteners concentrations        |
|----------------|---------------------------------------------|
| R <sub>1</sub> | 0                                           |
| R <sub>2</sub> | ACE-100ppb                                  |
| R <sub>3</sub> | SUC-100ppb                                  |
| R <sub>4</sub> | CYC-100ppb                                  |
| R <sub>5</sub> | SAC-100ppb                                  |
| R <sub>6</sub> | ACE-100ppb+SUC-100ppb+CYC-100ppb+SAC-100ppb |

**Table S4. Batch Tests Design**

| Treatment | Reactor | Activated<br>sludge | ACE<br>(100ppb) | NaN <sub>3</sub> | aeration | Removal routes |
|-----------|---------|---------------------|-----------------|------------------|----------|----------------|
| 1         | R1      | +                   | +               | -                | +        | B+A+V+H        |
| 2         | R2      | +                   | +               | +                | +        | A+V+H          |
| 3         | R3      | -                   | +               | +                | +        | V+H            |
| 4         | R4      | -                   | +               | +                | -        | H              |

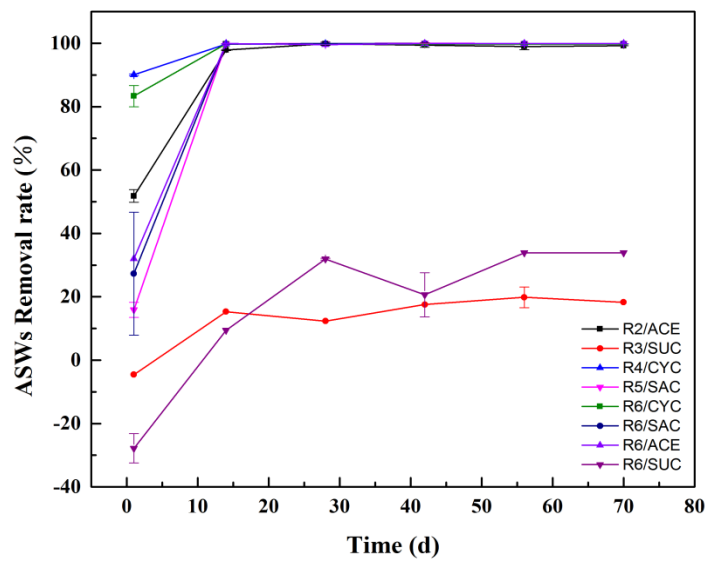
B: Biodegradation; A: Adsorption; V: Volatilization; H: Hydrolysis

**Table S5. Parameters for MRM acquisition of target ASs**

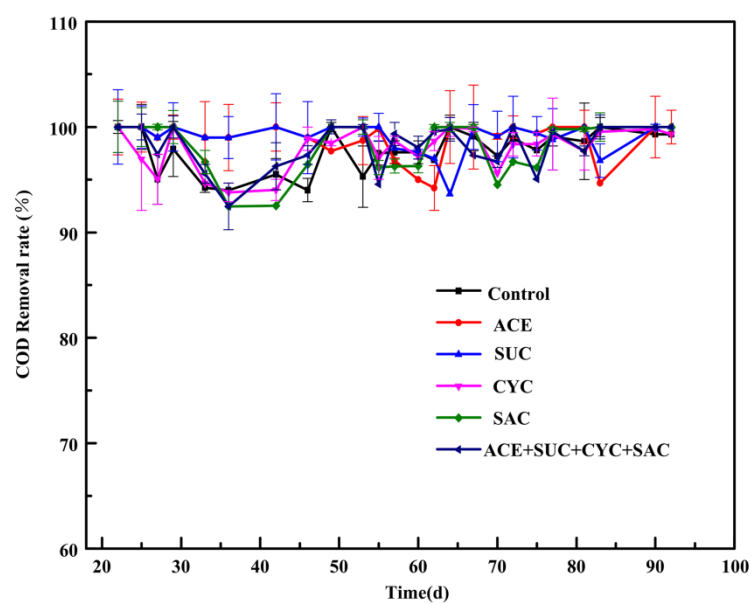
| Compound | Parent<br>(m/z) | Daughter (m/z) | Cone Voltage<br>(V) | Collision Energy<br>(V) |
|----------|-----------------|----------------|---------------------|-------------------------|
| ACE      | 162             | 82/78          | 38                  | 14/18                   |
| CYC      | 178             | 80             | 60                  | 28                      |
| SAC      | 182             | 106/42         | 55                  | 16/26                   |
| SUC      | 395             | 359/35         | 70                  | 10                      |

**Table S6. Instrumental and method validation data**

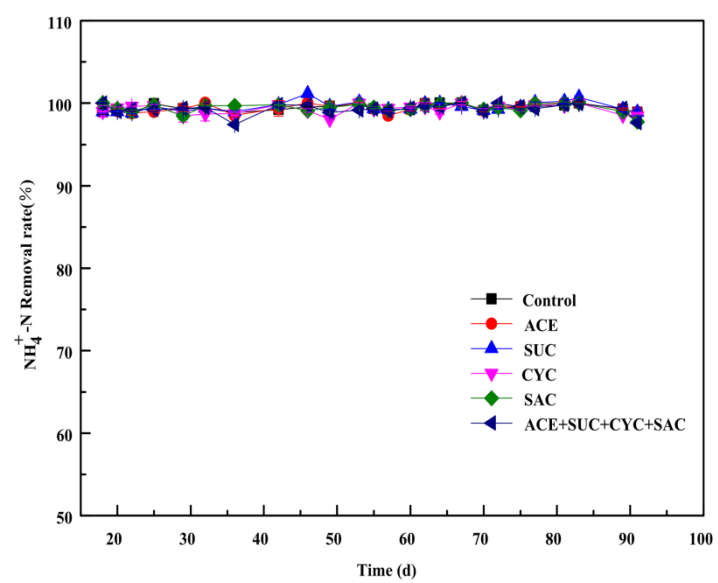
| Targeted<br>ASs | IDL<br>(ng L <sup>-1</sup> ) | MDL<br>(ng L <sup>-1</sup> ) | Recovery<br>Mean±RSD<br>(%) | Repeatability<br>RSD(%)(n=6) | Reproducibility<br>RSD(%)(n=6) |
|-----------------|------------------------------|------------------------------|-----------------------------|------------------------------|--------------------------------|
| ACE             | 10                           | 20                           | 89.91 ± 5.18                | 3.4                          | 9.6                            |
| CYC             | 10                           | 20                           | 93.17 ± 3.76                | 2.7                          | 10.4                           |
| SAC             | 10                           | 20                           | 92.16 ± 2.25                | 4.3                          | 11.3                           |
| SUC             | 50                           | 100                          | 93.73 ± 3.12                | 4.5                          | 7.2                            |



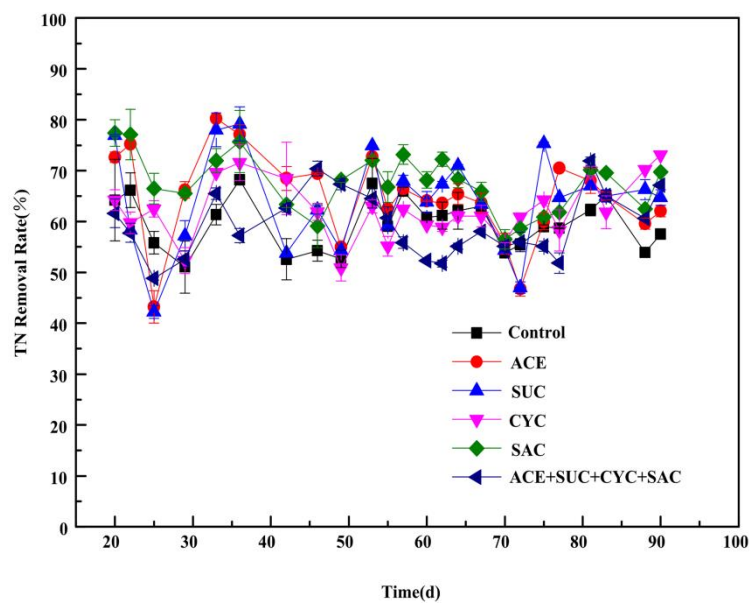
**Figure S1. ASWs removal in different samples during SBRs operation**



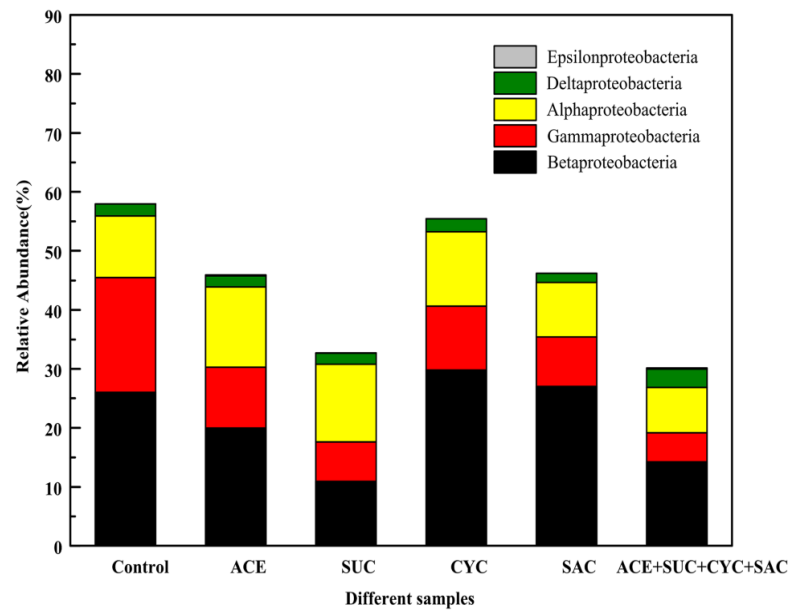
**Figure S2. COD removal in different samples during SBRs operation**



**Figure S3.  $\text{NH}_4^+\text{-N}$  removal in different samples during SBRs operation**



**Figure S4. TN removal in different samples during SBRs operation**



**Figure S5. Distribution of Proteobacteria in activated sludge samples**