

Cover sheet for SI

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Role of Extracellular Polymeric Substances in a Methane Based Membrane Biofilm Reactor Reducing Vanadate

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Table S1. Fluorescence spectral parameters of EPS in the biofilm of each stage determined by 3D-EEM.

| | Peak A (Tryptophan) | | | Peak B (Humic acid) | | | Peak C (aromatic protein-like substances) | | |
|----------|---------------------|-----|-----------|---------------------|-----|-----------|---|-----|-----------|
| | Ex | Em | Intensity | Ex | Em | Intensity | Ex | Em | Intensity |
| Inoculum | 280 | 305 | 2799 | - | - | - | - | - | - |
| Stage 1 | 290 | 335 | 2943 | 365 | 445 | 592 | 230 | 325 | 868 |
| Stage 2 | 290 | 345 | 3102 | 355 | 440 | 2046 | 230 | 335 | 707 |
| Stage 3 | 290 | 340 | 3177 | 365 | 445 | 969 | - | - | - |
| Stage 4 | 285 | 345 | 3229 | 355 | 440 | 2356 | 230 | 325 | 1016 |

Table S2. Absorbance of EPS in the biofilms of each stage measured by FTIR.

| | Inoculum | Stage 1 | stage 2 | stage 3 | stage 4 |
|------------------|----------|---------|---------|---------|---------|
| OH ⁻ | 0.410 | 0.482 | 0.828 | 0.419 | 0.968 |
| C-H | 0.145 | 0.159 | 0.447 | 0.178 | 0.457 |
| C=O | 0.256 | 0.305 | 0.655 | 0.249 | 0.636 |
| COO ⁻ | 0.104 | 0.131 | 0.392 | 0.135 | 0.375 |
| O-C-O | 0.175 | 0.186 | 0.504 | 0.195 | 0.481 |

Table S3. Pearson Correlation analysis of V(V) flux and predominant genus in the biofilms of all stages.

| | | V(V) flux | <i>Methylomonas</i> | <i>Methylophilus</i> | <i>Denitratisoma</i> | <i>Methylococcus</i> | <i>Ignavibacterium</i> | <i>Meiothermus</i> |
|------------------------|---------------------|-----------|---------------------|----------------------|----------------------|----------------------|------------------------|--------------------|
| V(V) flux | Pearson Correlation | 1 | .970* | .754 | .996** | .327 | .619 | -.280 |
| | Sig. (2-tailed) | | .030 | .246 | .004 | .673 | .381 | .720 |
| <i>Methylomonas</i> | Pearson Correlation | .970* | 1 | .681 | .987* | .090 | .758 | -.463 |
| | Sig. (2-tailed) | .030 | | .319 | .013 | .910 | .242 | .537 |
| <i>Methylophilus</i> | Pearson Correlation | .754 | .681 | 1 | .725 | .376 | .056 | -.382 |
| | Sig. (2-tailed) | .246 | .319 | | .275 | .624 | .944 | .618 |
| <i>Denitratisoma</i> | Pearson Correlation | .996** | .987* | .725 | 1 | .249 | .677 | -.337 |
| | Sig. (2-tailed) | .004 | .013 | .275 | | .751 | .323 | .663 |
| <i>Methylococcus</i> | Pearson Correlation | .327 | .090 | .376 | .249 | 1 | -.360 | .712 |
| | Sig. (2-tailed) | .673 | .910 | .624 | .751 | | .640 | .288 |
| <i>Ignavibacterium</i> | Pearson Correlation | .619 | .758 | .056 | .677 | -.360 | 1 | -.451 |
| | Sig. (2-tailed) | .381 | .242 | .944 | .323 | .640 | | .549 |
| <i>Meiothermus</i> | Pearson Correlation | -.280 | -.463 | -.382 | -.337 | .712 | -.451 | 1 |
| | Sig. (2-tailed) | .720 | .537 | .618 | .663 | .288 | .549 | |

*Significant correlation on the 0.05 level (2-tailed).

**Significant correlation on the 0.01 level (2-tailed).

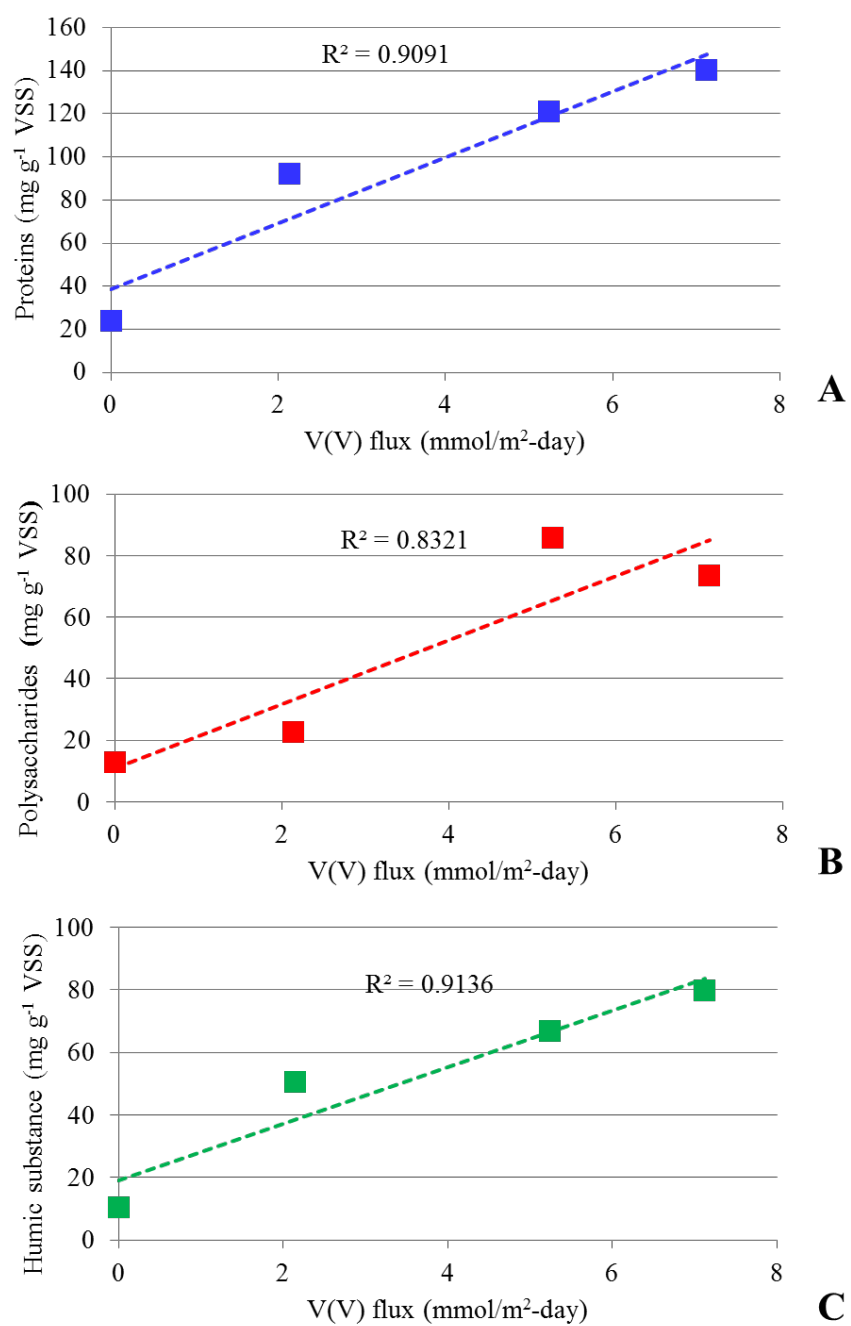


Figure S1. Linear relationship between V(V) flux and proteins (A), polysaccharides (B), and humic acid substances (C) in Stages 1, 2 and 4.

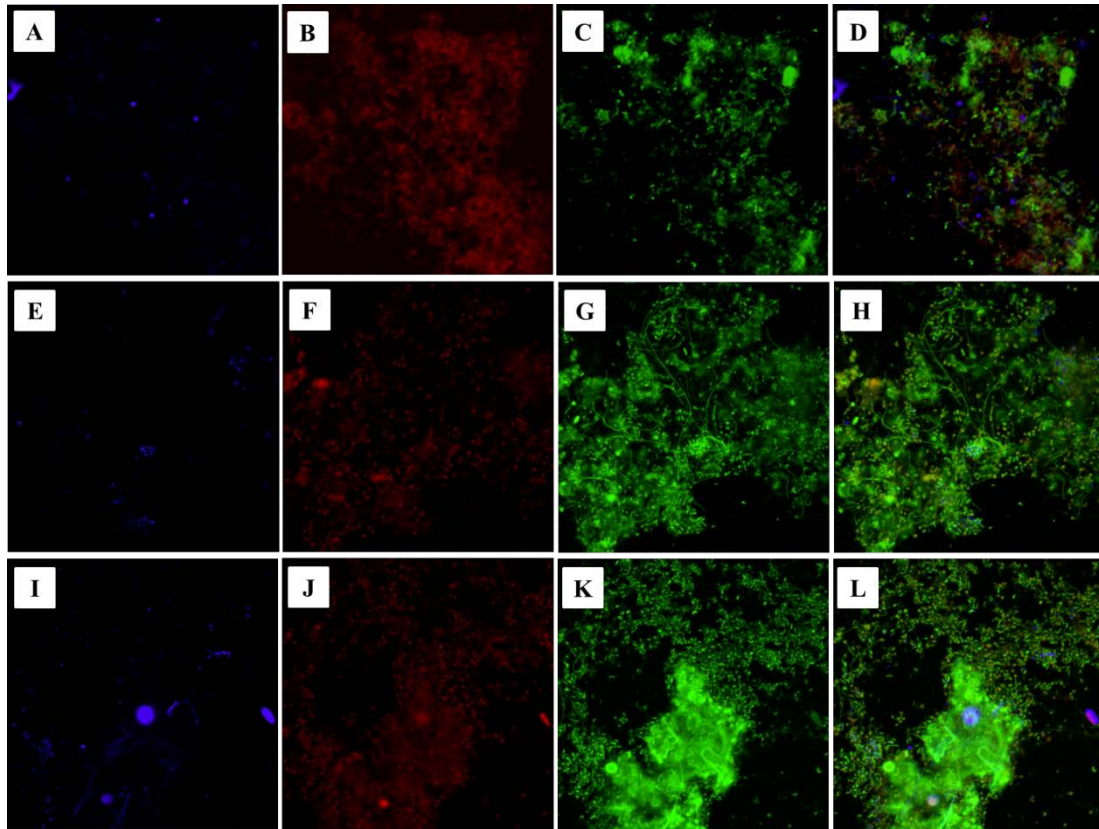


Figure S2. CLSM images targeting biofilms of Stage 1, 2 and 4. The spatial distribution of α -polysaccharides (A), β -polysaccharides (B), proteins (C), and combined image (D) of Stage 1; The spatial distribution of α -polysaccharides (E), β -polysaccharides (F), proteins (G), and combined image (H) of Stage 2; The spatial distribution of α -polysaccharides (I), β -polysaccharides (J), proteins (K), and combined image (L) of Stage 4.