

## Supplementary Material

### Effects of size and spacing of basalt fiber carrier media on performance, extracellular polymeric substances and microbial community of hybrid biological reactors

Qian Zhang <sup>a</sup>, Xianlin Liang <sup>a</sup>, Jing Wei <sup>a, b\*</sup>, Shanwei Li <sup>a, b</sup>, Xiang Xiao <sup>a</sup>, Zhigang Liu <sup>a, b</sup>,  
Xinshan Rong <sup>a, b</sup>, Zhishui Liang <sup>c</sup>, Zhiren Wu <sup>a, b, d\*</sup>

<sup>a</sup> School of the Environment and Safety Engineering, Jiangsu University, No. 301  
Xuefu Road, Jingkou District, Zhenjiang, Jiangsu 212013, China

<sup>b</sup> Institute of Environmental Health and Ecological Security, Jiangsu University, No.  
301 Xuefu Road, Jingkou District, Zhenjiang, Jiangsu 212013, China

<sup>c</sup> School of Civil Engineering, Southeast University, No. 2 Sipailou, Nanjing, Jiangsu  
210096, China

<sup>d</sup> ATK Holdings Group Co., Ltd, No. 68 Yigao Road, Gaocheng Town, Yixing,  
Jiangsu 214214, China

\*Corresponding authors: E-mail: [wuzhiren@ujs.edu.cn](mailto:wuzhiren@ujs.edu.cn) (Zhiren Wu);

[weijing@ujs.edu.cn](mailto:weijing@ujs.edu.cn) (Jing Wei)

**Number of pages: 9**

**Number of figures: 3**

**Number of tables: 4**

**Figure of contents:**

1. Illustration of collecting samples from frozen bio-nest for EPS and DNA extract analysis.
2. Rarefaction curves of samples at different depth of bio-nests with carrier size at 5.0 cm, 10.0 cm, 15.0 cm, 20.0 cm and 25.0 cm.
3. Rarefaction curves of samples at different depth of bio-nests with carrier spacing at 7.0 cm, 10.0 cm and 14.0 cm.

**Table of contents:**

1. Content and composition of extracted EPS from samples collected in the 1<sup>st</sup> experiment period.
2. The number of sequence and community diversity from each sample of bio-nest at different depth in the 2<sup>nd</sup> experimental period.
3. The number of sequence and community diversity from each sample of bio-nest at different depth in the 1<sup>st</sup> experimental period.
4. The number of sequence and community diversity from each sample of bio-nest at different depth in the 2<sup>nd</sup> experimental period.

**Fig. S1**

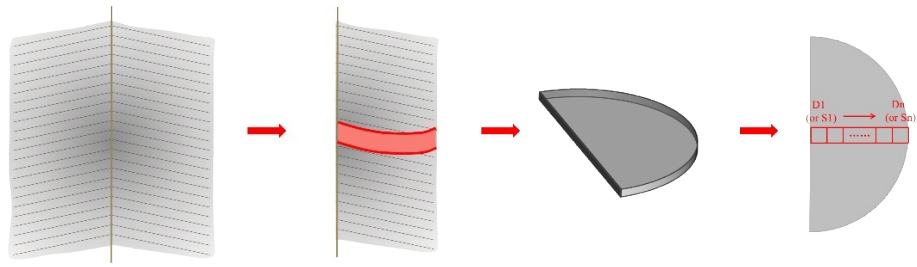


Fig. S1 Illustration of collecting samples from frozen bio-nest for EPS and DNA extract analysis

Fig. S2

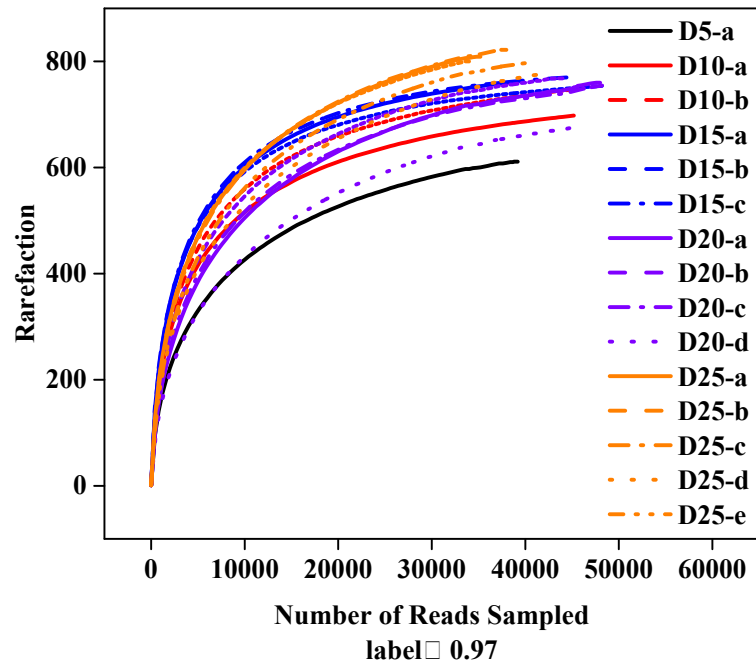


Fig. S2 Rarefaction curves of samples at different depth of bio-nests with carrier size at 5.0 cm, 10.0 cm, 15.0 cm, 20.0 cm and 25.0 cm

Fig. S3

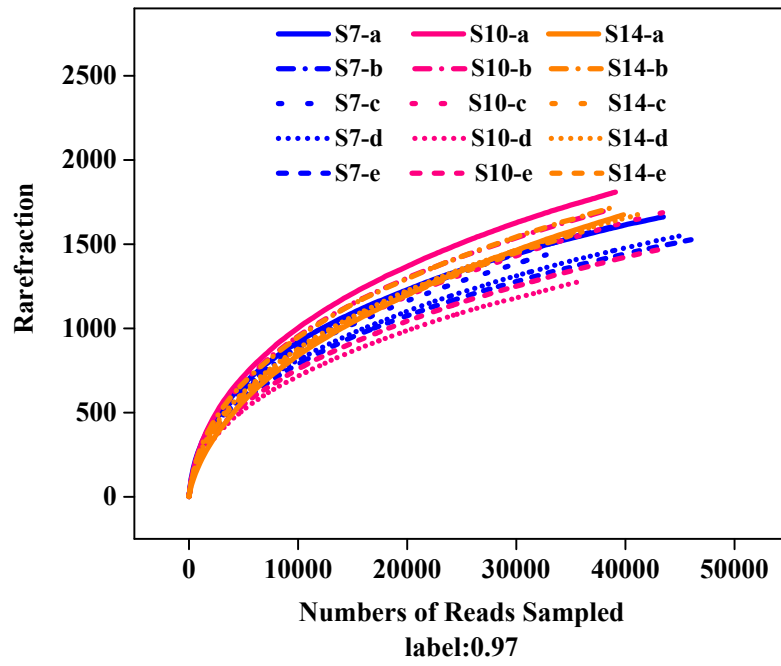


Fig. S3 Rarefaction curves of samples at different depth of bio-nests with carrier spacing at 7.0 cm, 10.0 cm and 14.0 cm.

**Table S1** Content and composition of extracted EPS from samples collected in the 1<sup>st</sup> experiment period.

| EPS composition<br>[ mg·g <sup>-1</sup> VSS ] |         | Sliced samples |      |      |      |      |      |      |      |       |      |      |      |      |      |      |  |
|---|---------|----------------|------|------|------|------|------|------|------|-------|------|------|------|------|------|------|--|
|   |         | D5             | D10  |      | D15  |      |      | D20  |      |       |      | D25  |      |      |      |      |  |
|   |         | 1              | 1    | 2    | 1    | 2    | 3    | 1    | 2    | 3     | 4    | 1    | 2    | 3    | 4    |      |  |
| S-EPS   | PN      | 50             | 47   | 34   | 13   | 18   | 63   | 72   | 86   | 108   | 89   | 24   | 78   | 85   | 94   | 85   |  |
|   | PS      | 64             | 44   | 24   | 12   | 24   | 47   | 44   | 42   | 41    | 56   | 15   | 36   | 38   | 42   | 49   |  |
|   | PN+PS   | 114            | 91   | 58   | 25   | 42   | 110  | 116  | 128  | 149   | 145  | 39   | 114  | 123  | 136  | 134  |  |
|   | Total   | 135            | 112  | 87   | 32   | 51   | 145  | 129  | 144  | 174   | 170  | 57   | 128  | 143  | 151  | 163  |  |
|   | PN/PS   | 0.78           | 1.07 | 1.42 | 1.08 | 0.75 | 1.34 | 1.64 | 2.05 | 2.63  | 1.59 | 1.60 | 2.17 | 2.24 | 2.24 | 1.73 |  |
|   | %S-EPS  | 0.35           | 0.40 | 0.25 | 0.15 | 0.20 | 0.31 | 0.41 | 0.46 | 0.49  | 0.43 | 0.35 | 0.39 | 0.37 | 0.42 | 0.43 |  |
| LB-EPS  | PN      | 34             | 29   | 13   | 20   | 28   | 44   | 15   | 9    | 7     | 3    | 13   | 14   | 22   | 24   | 27   |  |
|   | PS      | 36             | 14   | 6    | 10   | 19   | 20   | 17   | 9    | 9     | 5    | 10   | 13   | 15   | 11   | 26   |  |
|   | PN+PS   | 70             | 43   | 19   | 30   | 87   | 64   | 32   | 18   | 16    | 8    | 23   | 27   | 37   | 35   | 53   |  |
|   | Total   | 83             | 65   | 33   | 40   | 65   | 80   | 54   | 21   | 27    | 13   | 31   | 34   | 67   | 72   | 75   |  |
|   | PN/PS   | 0.94           | 2.07 | 2.17 | 2.00 | 1.47 | 2.20 | 0.88 | 1.00 | 0.78  | 0.60 | 1.30 | 1.08 | 1.47 | 2.18 | 1.04 |  |
|   | %LB-EPS | 0.21           | 0.23 | 0.09 | 0.19 | 0.25 | 0.17 | 0.17 | 0.07 | 0.08  | 0.03 | 0.19 | 0.10 | 0.17 | 0.20 | 0.20 |  |
| TB-EPS  | PN      | 83             | 60   | 116  | 70   | 79   | 112  | 54   | 58   | 53    | 102  | 29   | 53   | 56   | 44   | 42   |  |
|   | PS      | 55             | 31   | 86   | 49   | 55   | 89   | 60   | 56   | 67    | 84   | 38   | 78   | 83   | 71   | 76   |  |
|   | PN+PS   | 138            | 91   | 202  | 119  | 134  | 201  | 114  | 114  | 120   | 186  | 67   | 131  | 139  | 115  | 118  |  |
|   | Total   | 173            | 103  | 235  | 141  | 145  | 248  | 134  | 146  | 153   | 214  | 74   | 169  | 175  | 136  | 140  |  |
|   | PN/PS   | 1.51           | 1.94 | 1.35 | 1.43 | 1.44 | 1.26 | 0.90 | 1.04 | 0.79  | 1.21 | 0.76 | 0.68 | 0.67 | 0.62 | 0.55 |  |
|   | %TB-EPS | 0.44           | 0.37 | 0.66 | 0.66 | 0.56 | 0.52 | 0.42 | 0.47 | 0.43  | 0.54 | 0.46 | 0.51 | 0.45 | 0.38 | 0.37 |  |
| Total EPS                                     | PN      | 167            | 136  | 163  | 103  | 125  | 219  | 141  | 153  | 168   | 194  | 66   | 145  | 163  | 162  | 154  |  |
|   | PS      | 155            | 89   | 116  | 71   | 98   | 156  | 121  | 107  | 117   | 145  | 63   | 127  | 136  | 124  | 151  |  |
|   | PN+PS   | 322            | 225  | 279  | 174  | 223  | 375  | 262  | 260  | 285   | 339  | 129  | 272  | 299  | 286  | 305  |  |
|   | Total   | 391            | 280  | 355  | 213  | 261  | 473  | 317  | 311  | 354   | 397  | 162  | 331  | 385  | 359  | 378  |  |
|   | PN/PS   | 1.08           | 1.53 | 1.41 | 1.45 | 1.28 | 1.40 | 1.17 | 1.43 | 1.44  | 1.34 | 1.05 | 1.14 | 1.20 | 1.31 | 1.02 |  |
| TBEPS/LBEPS                                   | 2.08    | 1.58           | 7.12 | 3.53 | 2.23 | 3.10 | 2.48 | 6.95 | 5.67 | 16.46 | 2.39 | 4.97 | 2.61 | 1.89 | 1.87 |      |  |

**Table S2** The number of sequence and community diversity from each sample of bio-nest at different depth in the 2<sup>nd</sup> experimental period.

| EPS composition<br>   mg·g <sup>-1</sup> VSS |         | Sliced samples |      |      |      |      |       |       |       |       |       |       |       |       |       |       |
|--|---------|----------------|------|------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|  |         | S-7            |      |      |      |      | S-10  |       |       |       |       | S-14  |       |       |       |       |
|  |         | S7-1           | S7-2 | F7-3 | F7-4 | F7-5 | S10-1 | S10-2 | F10-3 | F10-4 | F10-5 | S14-1 | S14-2 | F14-3 | F14-4 | F14-5 |
| S-EPS  | PN      | 48             | 54   | 88   | 93   | 114  | 63    | 78    | 104   | 114   | 146   | 27    | 33    | 45    | 53    | 87    |
|  | PS      | 10             | 15   | 30   | 39   | 70   | 30    | 63    | 52    | 65    | 75    | 18    | 24    | 35    | 45    | 24    |
|  | PN+PS   | 58             | 69   | 118  | 132  | 184  | 93    | 141   | 156   | 179   | 221   | 45    | 57    | 80    | 98    | 111   |
|  | Total   | 73             | 84   | 126  | 173  | 233  | 127   | 185   | 196   | 217   | 261   | 60    | 83    | 92    | 116   | 124   |
|  | PN/PS   | 4.80           | 3.60 | 2.93 | 2.38 | 1.63 | 2.10  | 1.24  | 2.00  | 1.75  | 1.95  | 1.50  | 1.38  | 1.29  | 1.18  | 3.63  |
|  | %S-EPS  | 0.40           | 0.35 | 0.43 | 0.43 | 0.39 | 0.52  | 0.58  | 0.50  | 0.47  | 0.48  | 0.26  | 0.30  | 0.28  | 0.29  | 0.27  |
| LB-EPS                                       | PN      | 29             | 44   | 60   | 60   | 92   | 12    | 25    | 36    | 70    | 72    | 12    | 18    | 32    | 50    | 70    |
|  | PS      | 6              | 22   | 21   | 30   | 35   | 6     | 18    | 25    | 35    | 50    | 8     | 24    | 30    | 36    | 49    |
|  | PN+PS   | 35             | 66   | 81   | 90   | 127  | 18    | 43    | 61    | 105   | 122   | 20    | 42    | 62    | 86    | 119   |
|  | Total   | 57             | 87   | 96   | 108  | 163  | 39    | 49    | 84    | 134   | 140   | 36    | 54    | 88    | 106   | 142   |
|  | PN/PS   | 4.83           | 2.00 | 2.86 | 2.00 | 2.63 | 2.00  | 1.39  | 1.44  | 2.00  | 1.44  | 1.50  | 0.75  | 1.07  | 1.39  | 1.43  |
|  | %LB-EPS | 0.31           | 0.37 | 0.33 | 0.27 | 0.27 | 0.16  | 0.15  | 0.22  | 0.29  | 0.26  | 0.15  | 0.19  | 0.27  | 0.27  | 0.31  |
| TB-EPS                                       | PN      | 27             | 37   | 38   | 60   | 70   | 15    | 10    | 33    | 39    | 45    | 65    | 70    | 80    | 93    | 110   |
|  | PS      | 15             | 13   | 18   | 46   | 67   | 50    | 64    | 36    | 62    | 71    | 46    | 32    | 25    | 35    | 56    |
|  | PN+PS   | 42             | 50   | 56   | 106  | 137  | 65    | 74    | 69    | 101   | 116   | 111   | 102   | 105   | 128   | 166   |
|  | Total   | 51             | 67   | 71   | 120  | 199  | 79    | 84    | 109   | 111   | 139   | 139   | 141   | 145   | 178   | 192   |
|  | PN/PS   | 1.80           | 2.85 | 2.11 | 1.30 | 1.04 | 0.30  | 0.16  | 0.92  | 0.63  | 0.63  | 1.41  | 2.19  | 3.20  | 2.66  | 1.96  |
|  | %TB-EPS | 0.28           | 0.28 | 0.24 | 0.30 | 0.33 | 0.32  | 0.26  | 0.28  | 0.24  | 0.26  | 0.59  | 0.51  | 0.45  | 0.45  | 0.42  |
| Total EPS                                    | PN      | 104            | 135  | 186  | 213  | 276  | 90    | 113   | 173   | 223   | 263   | 104   | 121   | 157   | 196   | 267   |
|  | PS      | 31             | 50   | 69   | 115  | 172  | 86    | 145   | 113   | 162   | 196   | 72    | 80    | 90    | 116   | 129   |
|  | PN+PS   | 135            | 185  | 254  | 328  | 453  | 176   | 258   | 286   | 385   | 459   | 176   | 201   | 247   | 312   | 396   |
|  | Total   | 181            | 238  | 293  | 401  | 595  | 245   | 318   | 389   | 462   | 540   | 235   | 278   | 325   | 400   | 458   |
|  | PN/PS   | 3.35           | 2.70 | 2.70 | 1.85 | 1.60 | 1.05  | 0.78  | 1.53  | 1.38  | 1.34  | 1.44  | 1.51  | 1.74  | 1.69  | 2.07  |
| TBEPS/LBEPS                                  |         | 0.89           | 0.77 | 0.74 | 1.11 | 1.22 | 2.03  | 1.71  | 1.30  | 0.83  | 0.99  | 3.86  | 2.61  | 1.65  | 1.68  | 1.35  |

**Table S3** The number of sequence and community diversity from each sample of bio-nest at different depth in the 1<sup>st</sup> experimental period.

| Sample | Reads | OTU | Chao1      | ACE        | Shannon  |
|--------|-------|-----|------------|------------|----------|
| D5-a   | 35313 | 634 | 702.5698   | 707.0731   | 3.854578 |
| D10-a  | 55195 | 828 | 752.2174   | 746.662005 | 4.471216 |
| D10-b  | 55109 | 889 | 791.575    | 791.61576  | 4.366309 |
| D15-a  | 52972 | 901 | 798.038    | 799.806209 | 4.832881 |
| D15-b  | 62857 | 913 | 784.0545   | 779.401611 | 4.850527 |
| D15-c  | 54835 | 931 | 784.3784   | 788.163614 | 4.770873 |
| D20-a  | 42568 | 845 | 867.645161 | 855.100007 | 4.118644 |
| D20-b  | 37492 | 871 | 862.797872 | 857.836346 | 4.358815 |
| D20-c  | 56024 | 892 | 850.879121 | 850.075413 | 4.118702 |
| D20-d  | 40814 | 744 | 812.413043 | 800.549996 | 3.662508 |
| D25-a  | 32978 | 864 | 889.901639 | 906.22751  | 4.784613 |
| D25-b  | 32886 | 858 | 898.282828 | 893.256008 | 4.812928 |
| D25-c  | 35769 | 878 | 907.086207 | 914.590098 | 4.663805 |
| D25-d  | 38171 | 808 | 901.378378 | 915.951159 | 4.258644 |
| D25-e  | 38219 | 826 | 897.809524 | 896.708283 | 4.201706 |



**Table S4** The number of sequence and community diversity from each sample of bio-nest at different depth in the 2<sup>nd</sup> experimental period.

| Sample | Reads | OTU  | Chao1    | ACE      | Shannon  |
|--------|-------|------|----------|----------|----------|
| S7-a   | 43484 | 1662 | 2401.717 | 2807.979 | 5.135992 |
| S7-b   | 39576 | 1617 | 2591.171 | 3173.641 | 4.904461 |
| S7-c   | 32761 | 1437 | 2432.355 | 3025.386 | 4.730083 |
| S7-d   | 45367 | 1554 | 2302.414 | 2984.03  | 4.694634 |
| S7-e   | 46374 | 1531 | 2346.577 | 2880.471 | 4.259231 |
| S10-a  | 39087 | 1809 | 2713.243 | 3208.903 | 4.765271 |
| S10-b  | 38490 | 1703 | 2630.439 | 3164.148 | 4.69282  |
| S10-c  | 44691 | 1709 | 2680.113 | 3535.116 | 4.184476 |
| S10-d  | 35636 | 1275 | 2062.682 | 2886.719 | 3.896149 |
| S10-e  | 43440 | 1473 | 2574.953 | 3310.591 | 4.409277 |
| S14-a  | 39834 | 1674 | 2686.021 | 3437.517 | 3.364891 |
| S14-b  | 38716 | 1716 | 2738.832 | 3342.572 | 4.506304 |
| S14-c  | 33999 | 1549 | 2558.784 | 3269.686 | 4.005718 |
| S14-d  | 41260 | 1678 | 2605.773 | 3345.208 | 4.476093 |
| S14-e  | 36050 | 1573 | 2609.513 | 3273.387 | 4.264148 |