Electronic Supplementary Material (ESI) for Environmental Science: Nano.


# Single-cell metal-phenolic nanocoatings protect strictly anaerobic methanogen for methane production under atmospheric oxygen level

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This supporting information contains 15 pages with 9 figures and 4 tables.

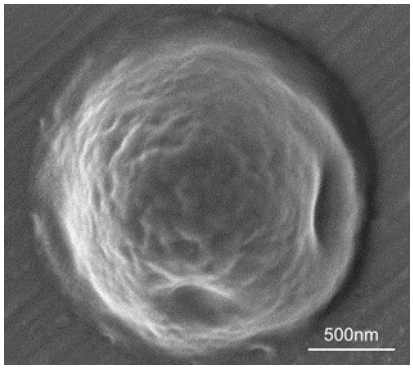


Figure S1. SEM images of *M. acetivorans* with 1.2 mM $FeCl_2$-$4H_2O$, 0.12 mM TA under anaerobic environment.

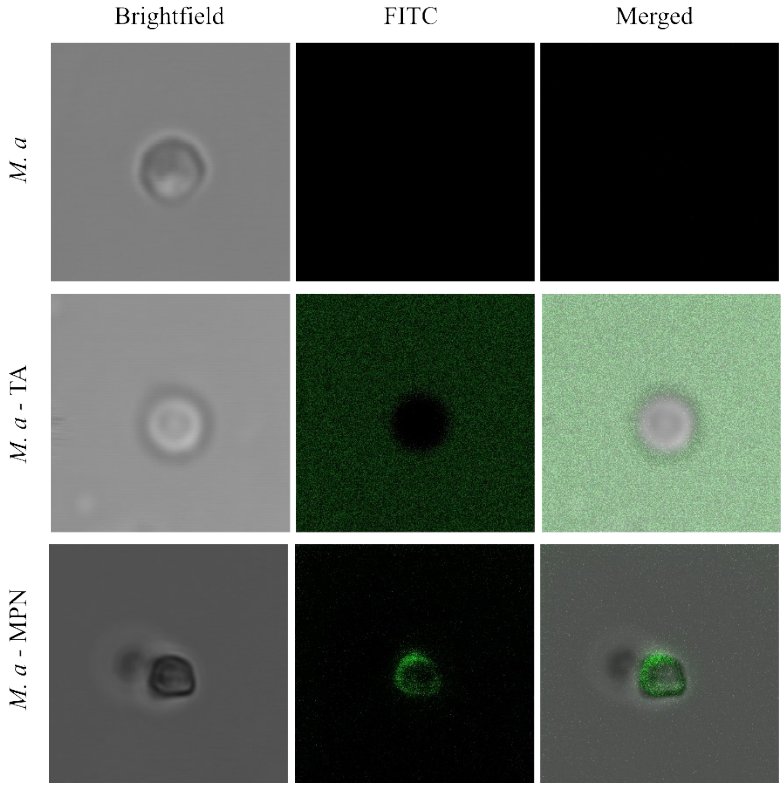


**Figure S2.** Confocal fluorescence microscopy bright-field and fluorescence images of native, FITC-labeled TA, FITC-labeled TA and $Fe^{2+}$ (MPN) assembled *M. acetivorans* (abbreviated as *M. a.* in the figure). TA cannot adsorb on the surface of *M. acetivorans* without $Fe^{2+}$, indicating that MPN formation on *M. acetivorans* requires the presence of both $Fe^{2+}$ and TA.

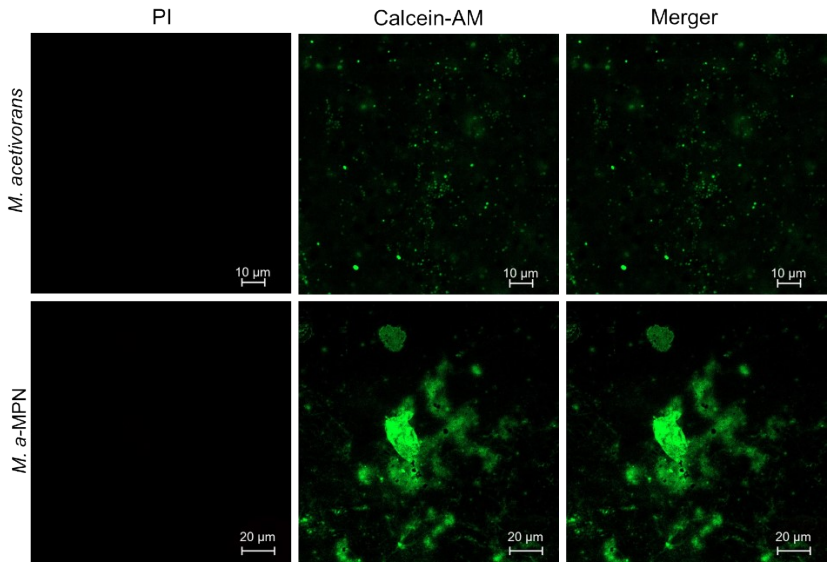


**Figure S3.** Confocal fluorescence microscopy images of *M. acetivorans* and MPN-coated *M. acetivorans* (*M. a*-MPN), depicting cell viability. Red fluorescence (PI dye) indicates dead cells, while green (Calcein-AM) signifies all cells.

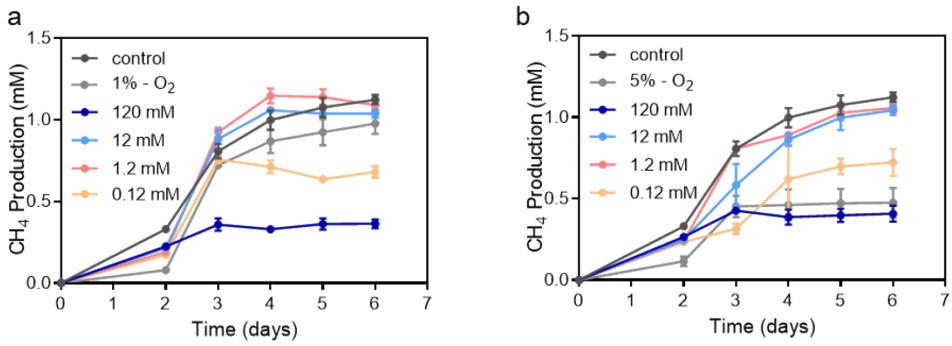


**Figure S4. *M. acetivorans*-coated MPN optimization.** Methane production profiles by MPN-coated *M. acetivorans*, with coatings prepared with $Fe^{2+}$ (0.12, 1.2, 12 and 120 mM) and TA (0.012, 0.12, 1.2 and 12 mM) under (a) 1% and (b) 5% oxygen. The methanogenesis of the MPN-coated *M. acetivorans* (MPN coating: 0.12 mM TA, 1.2 mM $FeCl_2 \cdot 4H_2O$) was not significantly different from that of the control group regardless of the oxygen concentration (1% or 5%). MPN coatings prepared at concentrations of 0.12 mM TA and 1.2 mM $FeCl_2 \cdot 4H_2O$ were used as the encapsulation material in subsequent experiments. Values are the mean of three biological replicates. Data are shown as the mean ± SD ($n$ = 5).

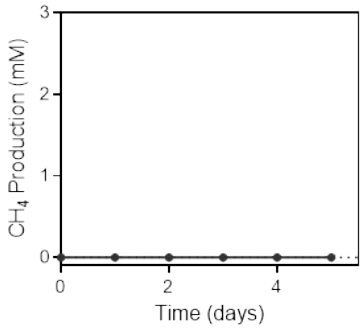


**Figure S5.** Methanogenic curve of MPN incubated in an anaerobic environment.

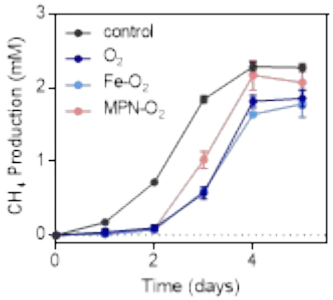


**Figure S6.** Methanogenic curves of *M. acetivorans* incubated in an anaerobic environment after 7 days of incubation under normal (anaerobic) (control), 21% oxygen ($O_2$), 1.2 mM $FeCl_2$ and 21% oxygen (Fe-$O_2$), and MPN (0.12 mM TA, 1.2 mM $FeCl_2 \cdot 4H_2O$) and 21% oxygen (MPN-$O_2$) conditions. Values are the mean of three biological replicates. Data are shown as the mean ± SD ($n$ = 5).

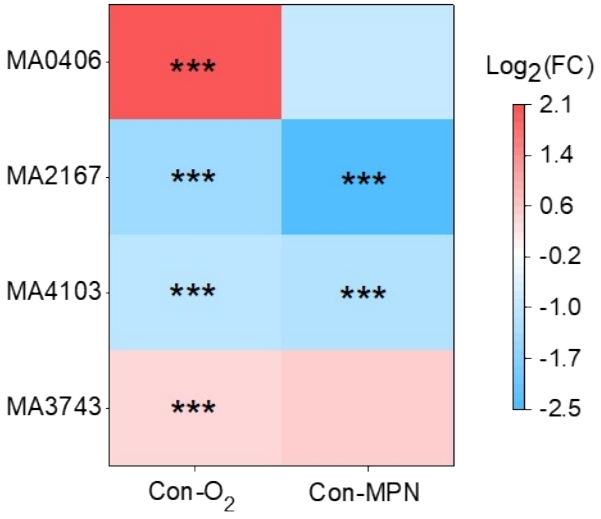


**Figure S7.** Transcript abundance analysis of methanogenic genes in *M. acetivorans* in 21% $O_2$ relative to anaerobic control (Con-$O_2$), and MPN-coated *M. acetivorans* in 21% $O_2$ relative to anaerobic control (Con-MPN), presented as $\log_2$(fold change) values. *$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001 (negative binomial distribution).

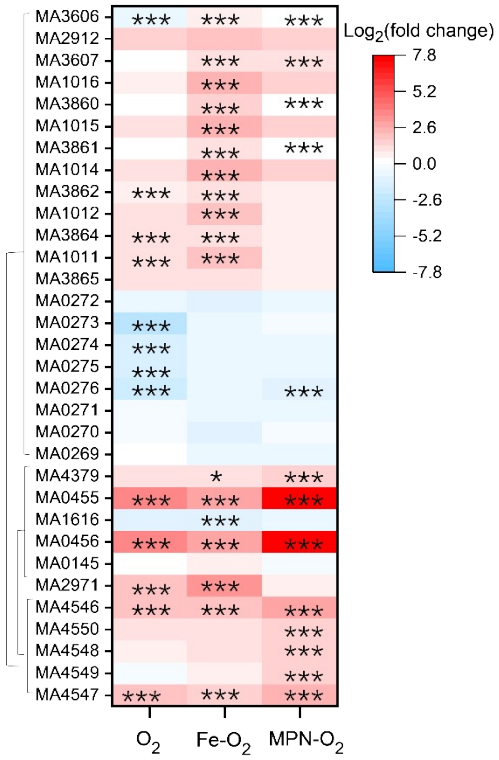


**Figure S8.** Transcript abundance analysis of methanogenic genes in *M. acetivorans* cultured for 3 days in 21% oxygen ($O_2$), 1.2 mM $FeCl_2$ and 21% oxygen (Fe-$O_2$), or MPN (0.12 mM TA, 1.2 mM $FeCl_2 \cdot 4H_2O$) and 21% oxygen (MPN-$O_2$), presented as $\log_2$(fold change) values. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (negative binomial distribution).

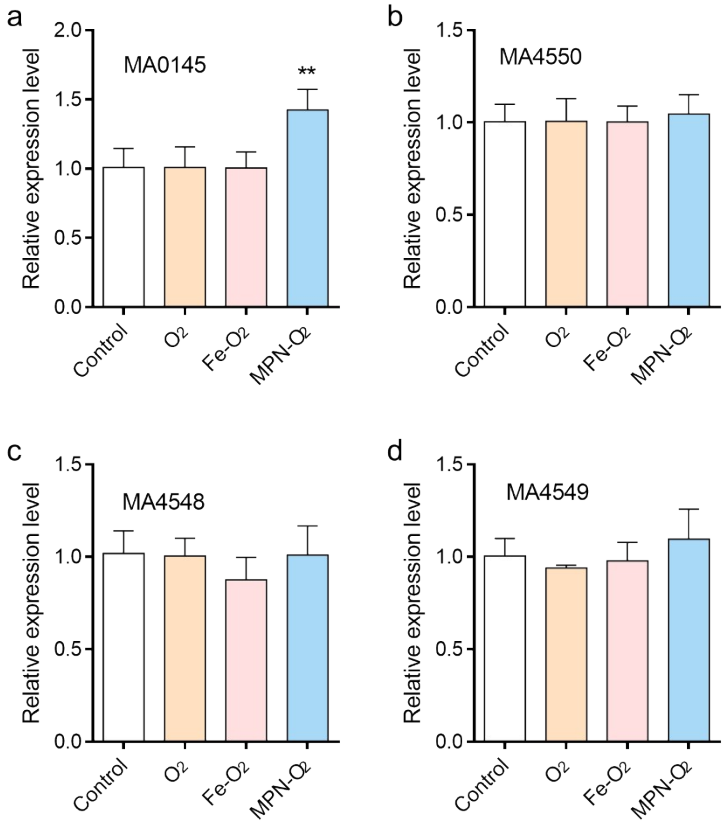


**Figure S9.** qPCR validation of methanogenesis metabolism-related genes (a) MA0145, (b) MA4550, (c) MA4548, and (d) MA4549 in *M. acetivorans* cultured for 2 days under normal (anaerobic) (Control), 21% oxygen ($O_2$), 1.2 mM $FeCl_2$ and 21% oxygen (Fe-$O_2$), or MPN (0.12 mM TA, 1.2 mM $FeCl_2 \cdot 4H_2O$) and 21% oxygen (MPN-$O_2$) conditions. Values are the mean of three biological replicates. Data are shown as the mean ± SD ($n = 3$), *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (two-tailed *t*-test).

**Table S1.** Composition of the anaerobic medium used for *M. acetivorans* culture

| Component | Concentration (g/L) |
|---|---|
| NaCl | 23.400 |
| $NaHCO_3$ | 4.000 |
| Yeast extract | 1.000 |
| $MgSO_4 \cdot 7H_2O$ | 9.450 |
| $Na_2HPO_4 \cdot 12H_2O$ | 1.510 |
| $NH_4Cl$ | 1.000 |
| KCl | 0.800 |
| $CaCl_2$ | 0.106 |
| Nitrilotriacetic acid | 1.500 |
| $MgSO_4 \cdot 7H_2O$ | 3.000 |
| $MnSO_4 \cdot H_2O$ | 0.500 |
| NaCl | 1.000 |
| $FeSO_4 \cdot 7H_2O$ | 0.100 |
| $CoSO_4 \cdot 7H_2O$ | 0.100 |
| $CaCl_2 \cdot 2H_2O$ | 0.100 |
| $ZnSO_4 \cdot 7H_2O$ | 0.100 |
| $CuSO_4 \cdot 5H_2O$ | 0.010 |
| $AlK(SO_4)_2$ | 0.010 |
| $H_3BO_3$ | 0.010 |
| $Na_2MoO_4 \cdot 2H_2O$ | 0.010 |

**Table S2.** Thickness of MPN films formed at different times for $O_2$ and methanol separation studies

| Time (h) | $O_2$ separation study | | | | Methanol separation study | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 14 | 0.5 | 1 | 2 | 12 |
| Thickness (nm) | 25 ± 4 | 48 ± 2 | 57 ± 4 | 157 ± 7 | 15 ± 1 | 16 ± 2 | 26 ± 4 | 142 ± 19 |

**Table S3.** Transcript analysis for genes identified in methanogenic metabolic pathway

| Locus tag | Gene name | $Log_2$(fold change) | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| | | $O_2$ | Fe-$O_2$ | MPN-$O_2$ | $O_2$ | Fe-$O_2$ | MPN-$O_2$ |
| MA3606 | AckA | -0.563471555 | 0.722207389 | 0.099228931 | 0.000222645 | 0.000488025 | 0.574970085 |
| MA2912 | Acsm3 | 1.540896511 | 2.089510428 | 1.613786086 | 9E-17 | 6.95E-15 | 1.44E-13 |
| MA1016 | CdhA1 | 0.822552025 | 2.450884118 | 1.465549063 | 7.96E-08 | 3.14E-25 | 2.28E-12 |
| MA3860 | CdhA2 | 0.104182662 | 1.330185667 | 0.408697394 | 0.569065996 | 3.43E-09 | 0.026174295 |
| MA1015 | CdhB1 | 1.071049515 | 2.364207674 | 1.65772676 | 0.000000042 | 3.83E-25 | 2.53E-13 |
| MA3861 | CdhB2 | 0.18842842 | 1.053562086 | 0.431857651 | 0.251005127 | 0.000000948 | 0.024607331 |
| MA1014 | CdhC1 | 0.938302858 | 2.401984094 | 1.550963431 | 1.61E-08 | 8.42E-23 | 2.55E-13 |
| MA3862 | CdhC2 | 0.610378654 | 1.039316878 | 0.516442156 | 0.000124931 | 0.0000034 | 0.00434895 |
| MA1012 | CdhD1 | 1.209248571 | 2.006765403 | 0.664670792 | 9.15E-15 | 1.86E-19 | 0.000975137 |
| MA3864 | CdhD2 | 0.890785447 | 1.03886697 | 0.571213354 | 9.81E-09 | 0.00000201 | 0.001429694 |
| MA1011 | CdhE1 | 1.22818701 | 1.942232696 | 0.552241895 | 7.95E-13 | 2.51E-18 | 0.006280797 |
| MA3865 | CdhE1 | 1.023396596 | 1.076517769 | 0.484849788 | 2.22E-10 | 0.00000103 | 0.00877315 |
| MA0272 | MtrA | -0.757144872 | -0.959753575 | -0.597236581 | 0.00000061 | 0.000815034 | 0.001311405 |
| MA0273 | MtrB | -2.632324963 | -0.637810907 | -0.432808801 | 5.27E-44 | 0.110732609 | 0.068961098 |
| MA0274 | MtrC | -1.624733411 | -0.75099505 | -0.433382665 | 3.29E-25 | 0.016464814 | 0.02511098 |
| MA0275 | MtrD | -1.419614577 | -0.772684655 | -0.600011352 | 9.35E-20 | 0.013158603 | 0.00138382 |
| MA0276 | MtrE | -1.923246723 | -0.659882041 | -1.255293456 | 5.13E-34 | 0.036341447 | 1.21E-10 |
| MA0271 | MtrF | -0.10296484 | -0.853116023 | -0.725350205 | 0.577338976 | 0.028291374 | 0.004760933 |
| MA0270 | MtrG | -0.368165079 | -0.919824395 | -0.426445834 | 0.13827599 | 0.010924336 | 0.072081631 |
| MA0269 | MtrH | 0.113023908 | -0.831108748 | -0.462087641 | 0.482500549 | 0.001009268 | 0.007408783 |
| MA4379 | MtaA | 0.996532505 | 1.1097553 | 1.424043752 | 5.16E-10 | 0.000000366 | 5.29E-14 |
| MA0455 | MtaB | 3.49457005 | 2.741394376 | 7.758330485 | 2E-86 | 2.79E-16 | 3.43E-220 |
| MA1616 | MtaB1 | -0.971260754 | -1.112445169 | -0.861890699 | 0.00000139 | 0.000158874 | 0.000121517 |
| MA0456 | MtaC | 3.703494974 | 2.715281367 | 7.786107409 | 1.48E-91 | 6.17E-17 | 1.1E-213 |
| MA0145 | MtmC1 | 0.100513737 | 0.698552752 | -0.160808743 | 0.588218066 | 0.005048935 | 0.526145703 |
| MA2971 | MtmC | 1.868896431 | 3.270518716 | 0.808425902 | 2.66E-16 | 3.99E-14 | 0.02705676 |
| MA4546 | McrA | 1.89587011 | 1.752963405 | 2.74845581 | 7.67E-26 | 2.97E-10 | 1.3E-35 |
| MA4550 | McrB | 0.968565084 | 0.878715954 | 1.389506794 | 7.17E-09 | 0.001852266 | 2.63E-12 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| MA4548 | McrC | 0.668057164 | 0.878810454 | 1.647918392 | 0.0000379 | 0.000642359 | 1.62E-19 |
| MA4549 | McrD | -0.270360511 | 0.794411823 | 1.338704035 | 0.091856609 | 0.002964413 | 7.22E-13 |
| MA4547 | McrG | 1.750885758 | 1.315501124 | 2.413948104 | 1.18E-21 | 0.0000115 | 1.56E-26 |
| MA2167 | Isf | -1.363878 | -0.756775 | -2.504374 | 1.48829E-11 | 0.00044 | 2.8016E-21 |
| MA0406 | Isf | 2.125658 | 1.374984 | -0.886991 | 1.3758E-07 | 0.00277 | 0.211682 |
| MA3743 | FprA | 0.400687 | 0.823335 | 0.480182 | 0.000958 | 6.94182E-05 | 0.062069 |
| MA4103 | \ | -0.995635 | -1.11847 | -1.153404 | 2.17531E-08 | 1.704E-07 | 4.48234E-07 |

**Table S4.** Primers used in this study[a]

| Primer name | Sequence (5′→3′) |
|---|---|
| Arc349f | GYGCASCAGKCGMGAAW |
| Arc806r | GGACTACVSGGGTATCTAAT |
| ma1616-qpcrf | TACGACTGTGCTCTGATGAATG |
| ma1616-qpcrr | CTATCGCCTCTCCTACTCTGTAA |
| ma4379-qpcrf | CACACCAACCCTGAACTGAT |
| ma4379-qpcrr | GACAAGGACAGTAAGGCAGTAG |
| ma0145-qpcrf | GAAGCTGCTGAGGTCTTTCTAC |
| ma0145-qpcrr | GGTTCCTTCCTCTCCTTCTACT |
| ma4550-qpcrf | AACGAAGGTCTTGGCTTCTC |
| ma4550-qpcrr | GATACCGGACTGCTCATAGATTG |
| ma4548-qpcrf | GTGAGTGTGCTTGTACTCTACTC |
| ma4548-qpcrr | CATTTCAATCTGTGCGACTTCTAC |
| ma4549-qpcrf | TTCAGATCGGAGATCAGGTTATTG |
| ma4549-qpcrr | GGAGCATCTTGTCGCATACT |

[a] "f" indicates forward primer and "r" indicates reverse primer. A one-letter code for degenerate bases is used according to the International Union of Biochemistry (IUB), where Y stands for C, T; K for G, T; S for C+G; W for A, T; and M for A, C.