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Title	Graphene facilitates biomethane production from protein derived glycine in anaerobic digestion
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Publication date	2018-11-22
Original Citation	Lin, R., Deng, C., Cheng, J., Xia, A., Lens, P. N. L., Jackson, S. A., Dobson, A. D. W. and Murphy, J. D. (2018) 'Graphene facilitates biomethane production from protein derived glycine in anaerobic digestion', iScience. 10, pp. 158-170. doi:10.1016/j.isci.2018.11.030
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.isci.2018.11.030
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Download date	2026-11-07 03:20:40
Item downloaded from	https://hdl.handle.net/10468/7195



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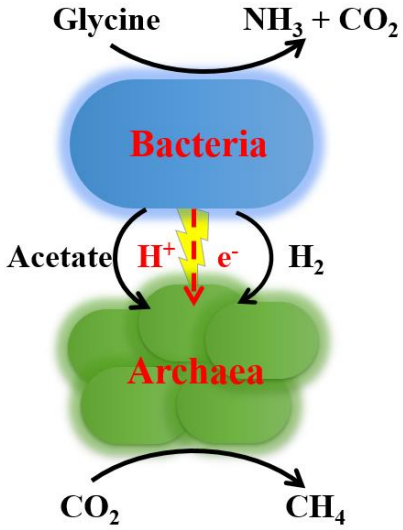
ISCI, Volume *10*

Supplemental Information

Graphene Facilitates Biomethane Production from Protein-Derived Glycine in Anaerobic Digestion

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Supporting Information



$$i = \sigma \bullet \frac{S_{conduit}}{d} \bullet (E_{met} - E_{ace})$$

i=Electric current associate with electron carrier flux

σ=Electrical conductivity of graphene

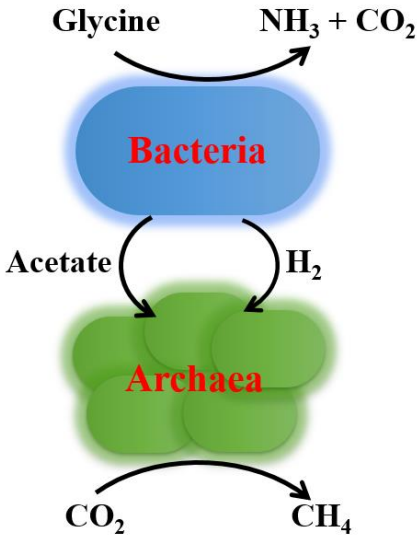
$S_{conduit}$=Cross-sectional area of the electron conduit

d=Distance between cells

E_{met}=Redox potential of ethanol oxidation reaction

E_{ace}=Redox potential of CO_2 reduction reaction

Figure S1. Calculation of maximum electron flux for graphene-based direct interspecies electron transfer (DIET) between acidogenic bacteria and methanogenic archaea, related to Table 2



$$i = D_f \bullet \frac{S_{cell}}{d} \bullet ([H_2]_{highest} - [H_2]_{lowest}) \bullet n \bullet F$$

i=Electric current associate with electron carrier flux

D_f=Diffusion coefficient of H_2 in water

S_{cell}=Surface area of the cells

d=Distance between cells

$[H_2]_{highest}$=Highest H_2 concentration produced by acetogen

$[H_2]_{lowest}$=Lowest H_2 concentration reached by methanogen

n=mol of electrons per mol of H_2

F=Faraday's constant

Figure. S2 Calculation of maximum electron flux for interspecies hydrogen transfer (MIET) between acidogenic bacteria and methanogenic archaea, related to Table 2

Transparent Methods

Inoculum

The inoculum for biomethane potential (BMP) assays was sourced from lab-scale continuous stirred-tank reactors, which are operated at 35 °C. The lab reactors were fed with various feedstocks such as seaweed, grass and animal slurry. The inoculum was kept at 35 °C in a water bath, while being fed once a week with cellulose as a carbon source (organic loading rate of 1.0 g/L/d). Prior to the BMP experiments, the inoculum was degassed for two weeks. The total solid (TS) content of the inoculum was 2.38 wwt%, and the volatile solid (VS) content was 1.34 wwt%.

Biomethane potential assays

Batch experiments of AD were carried out in triplicate in the AMPTS II system (Bioprocess Control, Sweden). The BMP system has the capacity to accommodate 15 glass fermenters (each has a total volume of 650 mL with a working volume of 400 mL). Five experimental groups were designed based on the additions of graphene (0, 0.25, 0.5, 1.0 and 2.0 g/L) in anaerobic digestion. In BMP assays, 150 mL of inoculum (containing 2.0 g VS) were added to each bottle. Subsequently, 1.0 g of amino acid glycine as the substrate were added to each glass fermenter to effect an inoculum to substrate ratio of 2:1. The final liquid volume in each bottle was adjusted to 400 mL with distilled water. Afterwards, all fermenter were sealed, purged with nitrogen gas for 5 min, and maintained at 35 °C in water bath. The biomethane volume was automatically recorded by the AMPST II system (Lin et al., 2018).

Analytic methods

The VS and TS content of the anaerobic inoculum was analyzed by drying of the sample for 24 h

at 105 °C. The ash content was calculated based on the method of subsequent heating for 2 h at 550 °C (Lin et al., 2018). The soluble metabolic products (mainly acetic acid) was characterized through a gas chromatography system (GC; Agilent 7890A, USA) equipped with a flame ionization detector and a DB-FFAP column (Lin et al., 2018). Before injecting to the GC, the liquid samples were first centrifuged at 5000 rpm for 5 min and then adjusted with orthophosphoric acid to a pH value of 2.0. All of the trials and measurements were conducted in triplicate.

To identify the microbial communities in response to different addition levels of graphene, the digestate samples were taken at the end of the digestion period and further analysed. The samples of digestates were rinsed with phosphate-buffered saline, centrifuged for 10 min at 4 °C, and then stored at −20 °C until further processing. The procedures of identifying the bacterial and archaeal communities were as follows. DNA was extracted following the manufacturer's protocol (Omega Bio-Tec, China). PCR amplicon libraries were generated using primers spanning the V3-V4 hypervariable region of the 16S rRNA gene. All PCR reactions from each sample were performed in duplicate in order to minimize bias. The products were checked on 2% agarose gels to determine the success of amplification. Duplicate amplicon samples were pooled together in equal proportions based on their molecular weight and DNA concentrations, purified using calibrated Ampure XP beads and sequenced using the Illumina Miseq platform (Illumina, USA) (Sangon Biotech Shanghai, China). Final operational taxonomic units (OTUs) were taxonomically classified using BLASTN against a curated database derived from RDP. The raw metagenomics datasets have been deposited into the NCBIs sequence Read Archive with the access number SRP158026.

Calculations

Understanding the kinetics of biomethane production is important when evaluating the digestion performance and designing the digester. Three types of models including first-order kinetic model, modified Gompertz model, and Cone model were employed to simulate and compare the kinetic patterns of the biomethane yield from digestion of glycine (Table 1). The kinetic parameters (such as P_m, R_m, k, and λ) were estimated by fitting the BMP data into the models via Origin 8.5 software. The Root Mean Square Prediction Error (RMSE) was calculated to evaluate the accuracy of each model. Statistical analysis of variance (one-way ANOVA) was carried out using Origin 8.5 software to test the impact of graphene addition on the biomethane production. The value of $p < 0.05$ was considered to be statistically significant.

The calculations of theoretical interspecies electron transfer via MIET and DIET are based on the models proposed in a previous study (Lin et al., 2017). The maximum electron transfer flux for graphene-based DIET was calculated using the Ohm's law and Nernst equation as described in Eq. 1:

$$i = \sigma \cdot \frac{S_{conduit}}{d} \cdot (E_{Met} - E_{Ace}) \quad (1)$$

where i is the electron transfer flux, σ is the electrical conductivity of graphene, $S_{conduit}$ is the cross sectional area of the electron conduit, d is the distance between cells, E_{Met} is the redox potential of the glycine oxidation reaction, and E_{Ace} is the redox potential of the carbon dioxide reduction reaction. $\Delta E = E_{Met} - E_{Ace}$ can be determined using the following Eq. 2:

$$\Delta E = E_{Met} - E_{Ace} = \frac{\Delta G'}{nF} \quad (2)$$

where ΔE (V) is the maximum redox potential of the overall reaction for glycine oxidation and carbon dioxide reduction, n is mole electron per reaction, and F is the Faraday's constant. $\Delta G'$ can be calculated according to Eq. 3:

$$\Delta G' = \Delta G^{0'} + RTln\frac{[Acetate]^{2/3} \cdot pCH_4^{1/12} \cdot pNH_3 \cdot pCO_2^{7/12}}{[Gly]} \quad (3)$$

where $\Delta G^{0'}$ (kJ/mol) is the standard Gibbs free energy change per reaction, R = 8.315 J/(mol·K), [Acetate] and [Gly] are the concentrations of acetic acid and glycine in the reaction, pCH_4, pNH_3 and pCO_2 are the concentrations of methane, ammonia and carbon dioxide in the reaction, and T is the reaction temperature.

To determine the maximum electron transfer flux via MIET, the Fick's diffusion law was used to calculate the rate of hydrogen diffusion from bacteria to methanogens (Eq. 4):

$$i = D_f \cdot \frac{S_{cell}}{d} \cdot ([H_2]_{highest} - [H_2]_{lowest}) \cdot n \cdot F \quad (4)$$

where i is the electron transfer flux, D_f is the diffusion constant of hydrogen in water, S_{cell} is the surface area of the cells, d is the distance of between cells, n is mole electron per reaction, F is Faraday's constant, $[H_2]_{highest}$ is the highest hydrogen concentration produced by acetogens, and $[H_2]_{lowest}$ is the lowest hydrogen concentration reached by methanogens. The highest and lowest hydrogen concentration was calculated in terms of the electron-donating reaction and electron-consuming reaction, respectively.

The following parameters were used to determine the thermodynamic values: Glycine concentration ([Gly]) of 7.52 mM, acetate concentration ([Acetate]) of 14.85 mM, CH_4 partial pressure (pCH_4) of 0.6 atm, CO_2 partial pressure (pCO_2) of 0.35 atm, and NH_3 partial pressure (pNH_3) of 0.04 atm. An interbacterial distance (d) of 0.5 μm was assumed with cells (both bacteria and archaea) having a cylindrical shape (assuming diameter = 0.5 μm, length = 2.5 μm). The electrical conductivity of graphene (σ) was determined typically as 850 S/cm. Graphene conduit is assumed as a cuboid shape with a thickness of 16 nm and a length of 2 μm.

The maximum driving force for direct electron transfer is given by the redox potential (ΔE =

$\Delta E_{met} - \Delta E_{ace}$) of the overall reaction ($C_2H_5NO_2 + 1/2H_2O \rightarrow 1/12CH_4 + 2/3CH_3COO^- + 2/3H^+ + NH_3 + 7/12CO_2$, $\Delta G^{0\prime} = -44.3$ kJ/mol). ΔE is determined by Nernst equation $\Delta E = -\Delta G'/nF$ (n = mole electron per reaction, F is Faraday's constant), where $\Delta G'$ can be calculated according to Eq. S1:

$$\Delta G' = \Delta G^{0\prime} + RTln\frac{[Acetate]^{2/3} \cdot pCH_4^{1/12} \cdot pNH_3 \cdot pCO_2^{7/12}}{[Gly]} \quad \text{(Eq. S1)}$$

In which $R = 0.00831451$ kJ/mol/K, and $T = 308.15$ K. The calculated $\Delta G' = -48.92$ kJ/mol. By using Nernst equation, ΔE can be calculated as 0.761 V. By further using equation in Fig. S1, the maximum electron transfer flux of DIET can be obtained as 4.1×10^{-3} A.

To estimate the maximum electron transfer flux in MIET, the concentrations of reactants and products and boundary conditions were set identical to those in DIET. The diffusion constant of H_2 in water at 35 °C was determined as 5.9×10^{-5} cm^2/ s. Fick's diffusion law is used to compute the rate of H_2 diffusion from bacteria to archaea. The highest H_2 concentration was calculated in terms of the electron-donating reaction ($C_2H_5NO_2 + 2/3H_2O \rightarrow 2/3CH_3COO^- + 2/3H^+ + NH_3 + 2/3CO_2 + 1/3H_2$, corresponding to $\Delta G' = 0$). The highest hydrogen partial pressure in practice can be achieved as 1 mM (Stams et al., 2006).

In a similar way, the lowest H_2 concentration was calculated in terms of the electron-consuming reaction ($1/3H_2 + 1/12CO_2 \rightarrow 1/12CH_4 + 1/6H_2O$, corresponding to $\Delta G' = 0$). The lowest hydrogen partial pressure was derived as 3.476×10^{-6} atm, corresponding to 2.1 nM. By further using equation in Fig. S2, a maximum H_2 flux of approximately 9.8×10^{-9} A can be obtained.

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