Supplementary information

Response of *Escherichia coli* to hydrogen nanobubbles: an in vitro evaluation using synchrotron infrared spectroscopy

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Material and methods

1. Production of nanobubbles

H_2 NBs were acquired through electrolyzing method using the hydrogen-producing equipment, with the hydrogen concentration of about 2600 ppb in the water, purchased from *Nanobubble* company (Shanghai, China). N_2 NBs were produced through pressurizing nitrogen into the dd-water and followed by depressurizing. The concentration and size distribution of NBs were measured by Nanoparticle Tracking Analysis (NTA). dd-water (18.2 MΩ, produced by USF-ELGA Maxima water purification system) was used as control throughout the experiment.

2. Bacterial culture and treatments

E. coli (CGMCC 1.2463) was inoculated into 5 mL LB broth medium and incubated at 37 ℃ with continuous shaking (200 rpm) until logarithmic phase. The bacteria were collected and washed by Tris buffer solution (TBS; T5912, Sigma-Aldrich) for three times and diluted to a final concentration of approximately 10^8 CFU/mL for the following experiments.

In bubble water treatment groups, *E. coli* was incubated with hydrogen-rich water and nitrogen-rich water, respectively. In tannins treatment group, *E. coli* was incubated with tannins water solution which had a final concentration of 0.5 g/L. dd-water mixed *E. coli* was taken as non-treatment group. All the groups of bacteria were incubated at 37 °C for 5 h.

3. Bacterial growth curve assay

After incubation, all the bacterial samples were diluted 10 times using TBS, then 100 μL of each diluted sample was inoculated into 900 μL LB broth medium. Finally, 200 μL of each group was transferred into a 96-well plate, a microplate reader

(VERSA max microplate reader) was used to monitor their optical density variations at 600 nm.

4. Synchrotron FTIR spectroscopy measurement

The FTIR spectroscopy experiment was carried out at the beamline BL01B of Shanghai Synchrotron Radiation Facility (SSRF). The sample testing procedures were as detailed described in our previous works[1-3], briefly, the aperture was set to 20 μm×20 μm, the absorption spectra were collected by transmission mode within the wavenumber range 4000–650 cm^{-1} at a resolution of 4 cm^{-1} with 64 co-added scans. 9-point smoothing, automatic baseline correction, normalization and second-derivation correction were calculated using OMNIC 9.2. PCA was analyzed on MATLAB R2014a.

References

[1] Y.D. Wang, X.L. Li, Z.X. Liu, X.X. Zhang, J. Hu, J.H. Lü, Discrimination of foodborne pathogenic bacteria using synchrotron FTIR microspectroscopy, Nucl. Sci. Tech., 28 (2017) 49.

[2] Y.D. Wang, X.L. Li, J. Hu, J.H. Lü, Synchrotron infrared spectral regions as signatures for foodborne bacterial typing, Nucl. Sci. Tech., 30 (2019) 25.

[3] Y.D. Wang, W.T. Dai, Y. Wang, J.X. Liu, Z.X. Liu, Y.Y. Li, X.L. Li, J. Hu, J.H. Lü, How many cells are enough for single-cell infrared spectroscopy?, Chem. Commun., 56 (2020) 3773-3776.

Figures:

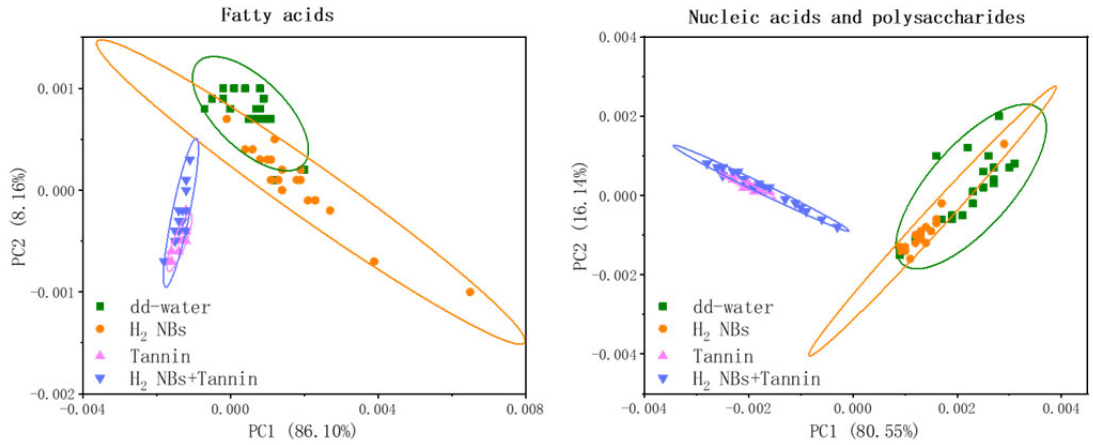

Fig. S1 ***E. coli*** **were incubated with dd-water (green), H_2 NBs (orange), tannin (0.5 g/L) (purple) and H_2 NBs+tannin (0.5 g/L) (blue) for 5 h, respectively. PCA on the second derivative spectra of the 4 groups of bacteria in the fatty acid region (3000–2800 and 1480–1340 cm^{-1}) and nucleic acid & polysaccharide region (1350–1000 cm^{-1}).**

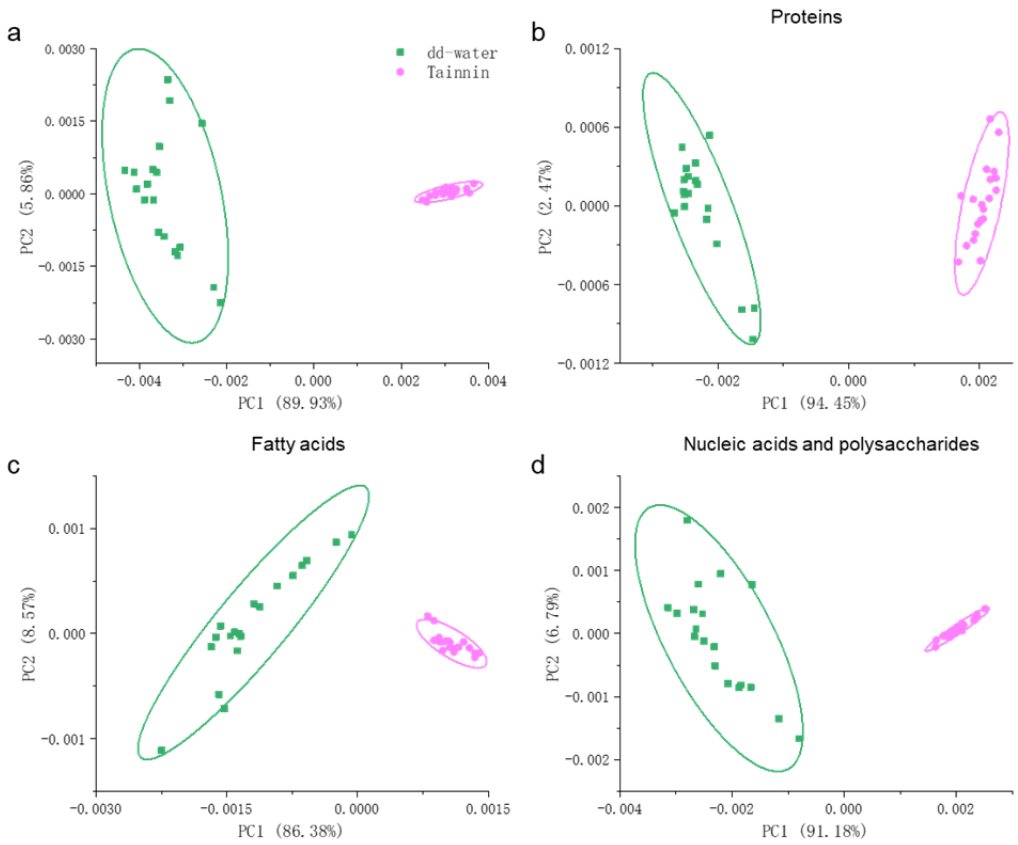

Fig. S2 PCA on the second derivative spectra of untreated (green) and 0.5 g/L tannin-treated (purple) *E. coli*. Full spectral region (a), protein region (b), fatty acid region (c) and nucleic acid & polysaccharide region (d) were analyzed, respectively.

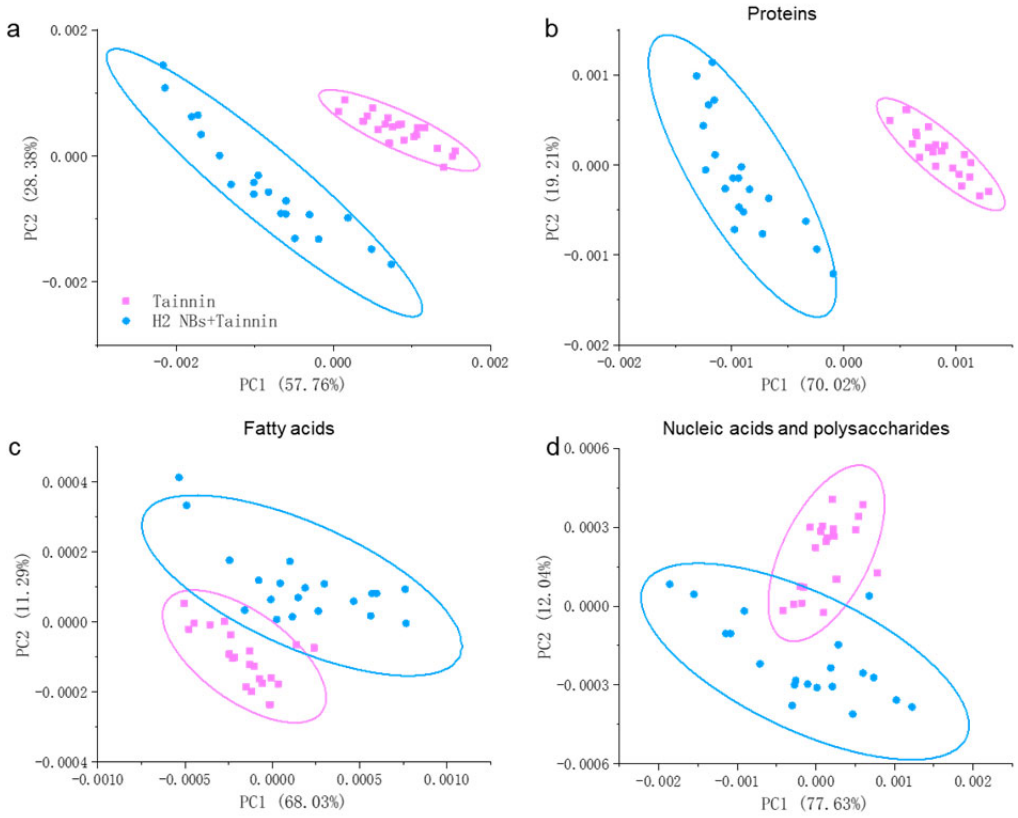

Fig. S3 PCA on the second derivative spectra of 0.5 g/L tannin-treated (purple) and H_2 NBs+0.5 g/L tannin treated (blue) *E. coli*. Full spectral region (a), protein region (b), fatty acid region (c) and nucleic acid & polysaccharide region (d) were analyzed, respectively.