**Supplementary Data (Journal:** **Environmental Technology)**

**Response of an aerobic denitrifier to Titanium dioxide nanoparticles exposure**

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**Materials and Methods**

**Characterization of TiO2 nanoparticles (NPs)**

TiO2 NPs (99.8% anatase) were purchased from Aladdin Biochemical Technology (Shanghai, China). Its TEM picture was exhibited in Fig. S1. It was shown that the particle size was ranged from 20 to 40 nm.



**Fig. S1** TEM picture for TiO2 NPs

**Measurements of bacterial cell growth**

Considering the formed aggregations between bacteria and TiO2 NPs, it seemed that OD600 was not quite fitted for the characterization of biomass. The consumption of ammonia nitrogen was used to depict the bacterial cell growth indirectly, as the ammonia nitrogen in the DM was primarily utilized for bacterial assimilation. First of all, the previous enriched bacterial cultures were inoculated into 150 mL DM in 250 mL conical flasks and cultivated at 30 ºC with a constant shaking speed of 150 rpm under aerobic conditions. Samples were taken at 4 h intervals, and then they were centrifuged to detect the bacterial dry weight. As a result, a standard curve (bacterial dry weight versus ammonia concentration) was obtained, which could be used to estimate the cell growth in this study.

**Measurements of N2O and O2 concentration**

Both N2O and O2 were measured by gas chromatography (GC-6890N, Agilent, USA) with an electron capture detector and a 5A molecular sieve column (30 m × 0.32 mm × 20 μm). N2 was used as the carrier gas with a flow rate of 3 mL min-1, and the temperatures of injector, oven and detector were 110, 200 and 300 ºC, respectively. Total N2O concentration including gaseous and dissolved N2O, please refer to Ma *et al*. (2016) for more details. Specifically, the concentrations of N2O in headspace were normalized to the liquid volume in serum bottles for comparable units. The dissolved N2O concentration was calculated from the balanced N2O partial pressure in the headspace using temperature-dependent solubility coefficient for N2O (Weiss and Price, 1980), as following equation:

                    *C* = Pi × K0

Where, *C* was the dissolved N2O concentration in the bottle, μg·L-1; Pi was the measured balanced N2O partial pressure in the headspace converted from parts-per-million (ppm) to partial pressure, atm; K0 was the volumetric solubility coefficient for N2O at 30 °C, 6.051×105 μg L-1 atm-1.

**Determination of nitrate removal rate**

Nitrate removal rates were determined using the following equation:

*R*= (*C0*-*C1*)/*t*

where *C0* was the initial nitrate concentration, mg/L; *C1* was the final concentration of nitrate (≈ 0), mg/L; *t* was the reaction time when nitrate concentration reached zero, h.

**Measurement of LDH release**

The LDH level was detected by a LDH Assay Kit (Jiancheng Bioengineering Co. Ltd., Nanjing, China) in accordance with the protocol specified by the manufacture. After exposed to TiO2 NPs for 8 h, the culture supernatants were obtained by centrifugation (10000 rpm for 5 min). Then the supernatant was treated according to the manufacturer’s instructions and measured at 450 nm absorbance via a microplate reader (Biotek, USA).

**Expressions of denitrification genes for strain *Pseudomonas stutzeri* PCN-1**

1. The normalized expression of each denitrification gene was calculated as follows:

Aerobic denitrification gene (*napA*, *nirS*, *cnorB* and *nosZ*, respectively) normalized expression (copies / copies) = aerobic denitrification gene expression level / V3 expression level

1. Relative fold change of denitrification gene expression was calculated with the following equation (Kapoor *et al*. 2015):

Relative fold change of aerobic denitrification gene expression = Log2 (*Tgene* / *Cgene*)

*Tgene*: the maximal normalized expression of aerobic denitrification genes in test sample (with TiO2 NPs exposure);

*Cgene*: the maximal normalized expression of aerobic denitrification genes in control sample (without TiO2 NPs exposure: 0 mg L-1 TiO2 NPs in the DM).

**Table S1** PCR primers of V3 region of 16S rDNA, *napA*, *nirS*, *cnorB* and *nosZ* for strain *Pseudomonas stutzeri* PCN-1.

|  |  |  |
| --- | --- | --- |
| Gene | Primers | Primer sequence (5’-3’) |
| *napA* | napA z3F | CGCGAACAAGCTGATGAAGG |
| napA z3R | AAGATCATCGGGATGTCGGC |
| *nirS* | nirS cd3aF | GTSAACGTSAAGGARACSGG |
| nirS R3cd | GASTTCGGRTGSGTCTTGA |
| *cnorB* | cnorB Z1F | CGTCGGTCAGATCCTCTTCG |
| cnorB Z1R | GCGATGATCACGTAGAGCCA |
| *nosZ* | nosZ 1527F | CGCTGTTCHTCGACAGYCA |
| nosZ 1773R | ATRTCGATCARCTGBTCGTT |
| V3 region of 16S rDNA | F341 | CCTACGGGAGGCAGCAG |
| R518 | ATTACCGCGGCTGCTGG |

**References**

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