

Supplementary Method.

The determination of Nir and Nar activity.

The activity of Nir and Nar was determined by ELISA Kits (Shanghai Enzyme-linked Biotechnology Co., Ltd.).

Sample pretreatment: The cell culture supernatant was collected by a sterile container, when secretory component was detected in the supernatant. The supernatant was centrifuged 20-min at the speed of 2000-3000 r.p.m. Collect supernatant again. The cell suspension was diluted with PBS (pH 7.2-7.4) and cell concentration reached 1 million/mL, when the composition of cells was detected. The cells were damaged by repeated freeze-thaw cycles to release intracellular components. Then the supernatant was collected after centrifugation 20-min at the speed of 2000-3000 r.p.m. If precipitation appeared, Centrifugal again.

Assay procedure: (1). Set Standard wells and testing sample wells. Add standard 50 μ L to standard well. (2). Set blank wells separately (blank comparison wells don't add sample and HRP-Conjugate reagent, other each step operation is same) and testing sample well. Add Sample dilution 40 μ L to testing sample well, then add testing sample 10 μ L (sample final dilution is 5-fold). Add sample to the bottom of wells. Don't touch the well wall as far as possible, and gently mix. (3). Add HRP-Conjugate reagent 100 μ L to each well, except blank well. (4). After closing plate with Closure plate membrane, incubate for 60 min at 37°C. (5). 20-fold wash solution was diluted 20-fold with distilled water and reserve. (6). Uncover Closure plate membrane, discard Liquid, dry by swing, add washing

buffer to every well and still for 30s then drain, repeat 5 times, dry by pat. (7). Add 50 μ L of Chromogen Solution A and Chromogen Solution B to each well, evade the light preservation for 15 min at 37°C. (8). Add Stop Solution 50 μ L to each well, Stop the reaction (the blue color change to yellow color). (9). Read absorbance of each well at 450nm, taking blank well as zero. Read should be carried after adding Stop Solution within 15min.

Supplementary Table

Table 1. The concentration of nitrite and nitrate in different positions of R2 and R3

Location (cm)	R2		R3	
	NO ₂ -N (mg/L)	NO ₃ -N (mg/L)	NO ₂ -N (mg/L)	NO ₃ -N (mg/L)
0	0	33.13±3.15	0.02±0.02	32.22±0.90
18	0.27±0.47	12.15±1.94	5.80±1.28	14.18±1.08
36	0	0.55±0.29	0.01±0.01	0.31±0.28

Note: The sampling position was from the bottom to the top of the sulfur packed bed.