Investigation of fate and behavior of tetracycline in nitrifying sludge system

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The blank experiment for TC elimination by hydrolysis and volatilization

Two reactors with only 10 mg/L and 1 mg/L TC were run with stirring at 90 rpm for 72 h in dark at room temperature. In the blank experiment, the elimination of TC was only accounted by hydrolysis and volatilization. As shown in Fig. S1, the concentration of TC remained almost constant during 72 h, indicating the elimination due to hydrolysis and volatilization was so slight to be ignored during the testing period.



Fig. S1 TC concentration profiles during the blank experiment with only TC

Fluorescent in situ hybridization (FISH) analyses

FISH was used to calculate the relative abundances of ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) with group specific probes. Samples taken at the beginning and at the end of operation in RI and RII were hybridized with three probes-pairs. The FISH images were analyzed to determine the percentage of NOB to the total bacteria and the relative amount of NOB to AOB.



Fig. S2 - Partial results of the image acquisition from FISH analysis. AOB is presented in red, while NOB appears in green. Bar = $20 \mu m$.

SEM analysis

The morphology of nitrifying sludge in RI and RII were observed with a scanning electron microscope (SEM) (JEOL JSM-7600F). The sludge samples were first fixed with 2% glutaraldehyde solution at 4°C for 12 h. The fixed sludge samples were dehydrated in graded water-ethanol solutions (50%, 70%, 80%, 90%, 95% and 100%), then lyophilized and sputter-coated with gold for SEM imaging.

As shown in Fig. S3, multilayer structure of sludge samples was observed in both reactors. The specific structure might protect bacteria inside from hazardous environment. It might explain the results that RI showed better nitrification performance and TC biodegradation than RII.



Fig. S3 SEM images of nitrifying sludge in RI (left) and RII (right)