



## A novel gas production bioassay of thiosulfate utilizing denitrifying bacteria (TUDB) for the toxicity assessment of heavy metals contaminated water

Ebenezer Ashun<sup>a</sup>, Woochang Kang<sup>a</sup>, Bhim Sen Thapa<sup>a</sup>, Anup Gurung<sup>a</sup>, Mostafa Rahimnejad<sup>b</sup>, Min Jang<sup>c</sup>, Byong-Hun Jeon<sup>d</sup>, Jung Rae Kim<sup>e</sup>, Sang-Eun Oh<sup>a,\*</sup>

<sup>a</sup> Department of Biological Environment, Kangwon National University, 192-1 Hoyoja-dong, Gangwon-do, Chuncheon-si, 200-701, Republic of Korea

<sup>b</sup> Biofuel and Renewable Energy Research Center, Chemical Engineering Department, Babol Noshirvani University of Technology, Babol, Islamic Republic of Iran

<sup>c</sup> Department of Environmental Engineering, Kwangju University, 20 Kwangjuon-Ro, Nowon-Gu, Seoul 01897, Republic of Korea

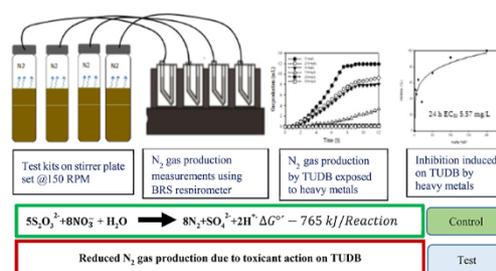
<sup>d</sup> Department of Earth Resources and Environmental Engineering, Hanyang University, Seoul 04763, Republic of Korea

<sup>e</sup> School of Chemical Engineering, Pusan National University, 63 Busandehak-ro, Geumjeong-Gu, Busan 46241, Republic of Korea

### HIGHLIGHTS

- A novel gas production bioassay of TUDB was developed.
- Gas production by TUDB was sensitive in detecting heavy metal-induced water toxicity.
- Gas production was inversely proportional to the concentration of heavy metals.
- Decreasing inhibitory order of the tested heavy metals was  $\text{Cr}^{6+} > \text{Cu}^{2+} > \text{Ag}^+ > \text{Ni}^{2+} > \text{As}^{3+} > \text{Cd}^{2+} > \text{Hg}^{2+} > \text{Pb}^{2+}$ .

### GRAPHICAL ABSTRACT



### ARTICLE INFO

Handling Editor: CHANG MIN PARK

#### Keywords:

Autotrophic denitrification  
Thiosulfate  
Bioassay  
Gas production  
Heavy metals  
Water toxicity

### ABSTRACT

This study reports for the first-time the possibility of deploying gas production by thiosulfate utilizing denitrifying bacteria (TUDB) as a proxy to evaluate water toxicity. The test relies on gas production by TUDB due to inhibited metabolic activity in the presence of toxicants. Gas production was measured using a bubble-type respirometer. Optimization studies indicated that 300 mg  $\text{NO}_3^- - \text{N/L}$ , 0.5 mL acclimated culture, and 2100 mg  $\text{S}_2\text{O}_8^{2-}/\text{L}$  were the ideal conditions facilitating the necessary volume of gas production for sensitive data generation. Determined  $\text{EC}_{50}$  values of the selected heavy metals were:  $\text{Cr}^{6+}$ , 0.51 mg/L;  $\text{Ag}^+$ , 2.90 mg/L;  $\text{Cu}^{2+}$ , 2.90 mg/L;  $\text{Ni}^{2+}$ , 3.60 mg/L;  $\text{As}^{3+}$ , 4.10 mg/L;  $\text{Cd}^{2+}$ , 5.56 mg/L;  $\text{Hg}^{2+}$ , 8.06 mg/L; and  $\text{Pb}^{2+}$ , 19.3 mg/L. The advantages of this method include operational simplicity through the elimination of cumbersome preprocessing procedures which are used to eliminate interferences caused by turbidity when the toxicity of turbid samples is determined via spectrophotometry.

\* Corresponding author.

E-mail address: [ohsangeun@kangwon.ac.kr](mailto:ohsangeun@kangwon.ac.kr) (S.-E. Oh).