Free nitrous acid controls sulfide and methane production in rising main sewers

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Keywords: Free nitrous acid; sulfide; methane

Introduction

Nitrite was employed to tackle odor, health, and other detrimental problems caused by biogenic hydrogen sulfide in many scenarios, e.g. swine manure, souring control in oil fields, wastewater treatment, municipal solid waste degradation (Cirne et al., 2008; Kaster and Voordouw, 2006; Okabe et al., 2003; Vigneron et al., 2007). In sewers, nitrite injection was shown to be effective in inhibiting sulfide and methane production in previous research (Jiang et al., 2010; Mohanakrishnan et al., 2008).

Nitrite, similar to molybdate, is classified as a metabolic inhibitor of sulfate-reducing bacteria due to its specific inhibition on the dissimilatory sulfate reduction process (Greene et al., 2006). Nitrite is an analog of sulfite in terms of both size and charge. Also, both the reduction of nitrite to ammonia and that of sulfite to sulfide are six-electron reductions. Nitrite therefore constitutes a competitive inhibitor of sulfite reductase, blocking the sulfate reduction metabolism (Einsiedl, 2009; Greene et al., 2003).

Free nitrous acid (FNA), the unionized form of nitrite, is generally formed when the pH is low. FNA has been shown to be the factor rather than nitrite itself for inhibiting denitrifying and phosphorus uptake processes (Vadivelu et al., 2006; Zhou et al., 2008). Besides, nitrous acid acts as a mutagen by deamination of the NH₂ group of adenine and/or cytosine to an ether group, thus altering their base pairing. FNA has been thus used to induce mutagenesis and this is lethal to cells without repair/correction (Klug and Cummings, 2000; Sidorkina et al., 1997).

Based on the inhibitory and toxic effects demonstrated by FNA, it was hypothesized that that FNA may be more effective in controlling sulfide and methane production by anaerobic sewer biofilms. The aim of this study is to investigate the possibility of achieving sulfide and methane control through short-term dosage of nitrite at a low pH, and to reveal the mechanisms involved.

Materials and methods

Four lab-scale sewer reactors, each with a volume of 0.75 L, were operated under anaerobic conditions, and fed with wastewater collected from a sewer pump station,
which primarily collects domestic wastewater (Jiang et al., 2009). The reactors stabilized in terms of sulfide and methane production rates in two months before sodium nitrite and chloric acid dosage to three of the reactors started. One reactor was used as the control, which did not receive nitrite or acid dosage. The other three were dosed nitrite at concentrations of 56, 112 and 222 mg-N per liter of wastewater, respectively, and acid to result in pH levels in wastewater at 5.6, 6.2, and 6.2, respectively. FNA can be calculated as FNA=NO$_2^-$-N/(K$_a$x10$^{pH}$), where K$_a$ is the ionization constant of the nitrous acid equilibrium equation. The value of K$_a$ is determined by K$_a$=e$^{-2300/(273+°C)}$. These gave rise to FNA concentrations of 0.36, 0.18 and 0.36 ppm-N, respectively.

Batch tests were conducted to determine the sulfide and methane production rates on selected days during the whole experimental period. Fresh sewage was pumped into the reactors. Wastewater samples were taken every 20 minutes. Sulfide and dissolved methane were analyzed by IC and GC (Jiang et al., 2010). The sulfide and methane production rates were thus determined by linear regression in the first hour with their concentrations respectively.

On day 0, biofilm samples were taken immediately before and after the dosing event. These biofilm samples were then stained with LIVE/DEAD® Baclight bacterial viability kits. Slides with stained biofilm were photographed under a confocal laser scanning microscope (Zeiss LSM 510 META), equipped with a Krypton-Argon laser (488 nm) and two He-Ne lasers (543 and 633 nm). Twenty images were quantified for each sample to calculate the cell death percentage.

**Results**

Both FNA levels succeeded in suppressing sulfide production and methane production. After stopping FNA dosage, SRB recovered to about 70-80% in 2 months. Meanwhile, methanogens only recovered their activity to 40-60%. No significant differences were found among the different FNA concentrations. This suggests that FNA dosage at 0.18 ppm for a duration of 24 hours is able to suppress/reduce both sulfide and methane in sewers for an extended period of time (weeks to months).
**Figure 1** Sulfide (A) and methane (B) production in the rising main sewer reactors were suppressed by FNA dosing, followed by a slow recovery after the cessation of dosage.

It was shown that FNA dosing had a lethal effect on sewer biofilm cells, resulting in very high cell death detected by LIVE/DEAD staining. Over 90% of cells have been deactivated in dosed reactors by the presence of FNA. FNA also caused biofilm dispersal. Detached biofilms were observed in the effluents from FNA-dosed reactors.

![Graph showing dead cells in biofilms (%)](image)

**Figure 2** FNA deactivated the sewer biofilm cells by 90% after the 24-hour exposure (upper). Two confocal microscopy images showed typical live (green) and dead (red) biofilm cells.

**Conclusions**

- FNA as low as 0.18 mg-N/L could suppress both sulfide and methane production after 24-hour of exposure. The suppression is followed by a slow recovery after stopping the FNA addition.

- FNA is toxic to the sewer biofilm cells. The biocidal effect is enormous under the experimental conditions.

**Acknowledgements**

The authors acknowledge the financial support provided by the Australian Research Council and many members of the Australian water industry through the Sewer Corrosion and Odour Research (SCORe) Project LP0882016 (for more details see: www.score.org.au). Guangming Jiang is grateful to the scholarships: Endeavour International Postgraduate Research Scholarship (IPRS) and University of Queensland International Living Allowance Scholarship (UQILAS).
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