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Impact of nitrate addition on biofilm properties and activities in rising main sewers

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ABSTRACT

Anaerobic sewer biofilm is a composite of many different microbial populations, including sulfate reducing bacteria (SRB), methanogens and heterotrophic bacteria. Nitrate addition to sewers in an attempt to control hydrogen sulfide concentrations affects the behaviour of these populations, which in turn impacts on wastewater characteristics. Experiments were carried out on a laboratory reactor system simulating a rising main to determine the impact of nitrate addition on the microbial activities of anaerobic sewer biofilm. Nitrate was added to the start of the rising main during sewage pump cycles at a concentration of 30 mg-N L^{-1} for over 5 months. While it reduced sulfide levels at the outlet of the system by 66%, nitrate was not toxic or inhibitory to SRB activity and did not affect the dominant SRB populations in the biofilm. Long-term nitrate addition in fact stimulated additional SRB activity in downstream biofilm. Nitrate addition also stimulated the activity of nitrate reducing, sulfide oxidizing bacteria that appeared to be primarily responsible for the prevention of sulfide build up in the wastewater in the presence of nitrate. A short adaptation period of three to four nitrate exposure events (approximately 10 h) was required to stimulate biological sulfide oxidation, beyond which no sulfide accumulation was observed under anoxic conditions. Nitrate addition effectively controlled methane concentrations in the wastewater. The nitrate uptake rate of the biofilm increased with repeated exposure to nitrate, which in turn increased the consumption of biodegradable COD in the wastewater. These results provide a comprehensive understanding of the impact of nitrate addition on wastewater composition and sewer biofilm microbial activities, which will facilitate optimization of nitrate dosing for effective sulfide control in rising main sewers.

1. Introduction

The presence of anaerobic conditions in sewer systems results in significant production of sulfide by sulfate reducing bacteria (SRB) present in the biofilm and solids sediment phases. Release of hydrogen sulfide from the liquid to the gas phase causes several detrimental effects including sewer corrosion, odour nuisance and health hazards. A number of operational strategies have been employed by the wastewater industry to minimize sulfide production in sewers. These range from simple

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Nomenclature

COD	chemical oxygen demand
DGGE	denaturing gradient gel electrophoresis
DO	dissolved oxygen
HRT	hydraulic retention time
IC	ion chromatography
NR-SOB	nitrate reducing, sulfide oxidizing bacteria
PCR	polymerase chain reaction
SAOB	sulfide anti-oxidant buffer
SE	standard error
SRB	sulfate reducing bacteria
VFA	volatile fatty acids

strategies like mechanical cleaning, to strategies that involve the addition of one or a combination of chemicals, such as nitrate, oxygen, metal salts (Fe^{2+}, Fe^{3+}, Zn^{2+}), alkali, chlorine, ozone or hydrogen peroxide, to more sophisticated strategies like the addition of SRB metabolic inhibitors or biocides (Boon, 1995; Boon et al., 1998).

Addition of a thermodynamically favourable electron acceptor like nitrate has been used over the last 70 years to control odours and sulfide production in many environments, including sewage systems and oil reservoirs (Bentzen et al., 1995; Heukelekian, 1943; Hubert and Voordouw, 2007; Jenneman et al., 1986; Mathioudakis et al., 2006; Zhang et al., 2008). Different nitrate salts (e.g. sodium and calcium nitrate) and different dosing concentrations have been trialled. Nitrate additions of 10 mg-N L^{-1} to wastewater successfully decreased sulfide concentrations from 4.2 mg L^{-1} to 0.2 mg L^{-1} in a 2.4 km long rising main (Bentzen et al., 1995). Saracevic et al. (2006) applied 40 mg-N L^{-1} nitrate to a 5.0 km long rising main sewer, and discovered that after a lag time of 3–4 days, nitrate reduced sulfide concentrations from 10–20 mg-S L^{-1} to below 2–3;mg-S L^{-1}. Nitrate concentrations of 5 mg-N L^{-1} in wastewater were reported to be sufficient to inhibit sulfide production in a 61 km long gravity sewer (Rodriguez-Gomez et al., 2005).

While there is conclusive evidence showing the effectiveness of nitrate addition on sulfide control, a full understanding of the mechanisms involved is still missing. A number of mechanisms have been described as potentially contributing to the inhibitory effects of nitrate: (i) nitrate addition can increase the redox potential of wastewaters and thus decrease sulfide production by SRB (Allen, 1949; Poduska and Anderson, 1981); (ii) prolonged inhibition has been attributed to the cytotoxic effect of products like nitrite (Eckford and Fedorak, 2004) or nitrous oxide (Jenneman et al., 1986), formed during nitrate reduction, on SRB metabolism; (iii) SRB activity is hindered by competition with nitrate reducing bacteria for organic electron donors (Hubert and Voordouw, 2007); and (iv) nitrate addition increases the pH of the wastewater due to denitrification (Rust et al., 2000), which in turn decreases sulfide production and stripping to the gas phase.

The goal of this study is to gain further understanding of the impacts of nitrate addition on wastewater composition and sewer biofilm microbial activities. In particular, the research aims to answer the following research questions:

1. Does nitrate addition inhibit the activity of SRB in sewer biofilm?
2. How does nitrate achieve effective sulfide control in sewers? Does nitrate addition stimulate sulfide oxidation coupled with nitrate reduction? Evidence has been produced in the last few years to demonstrate that nitrate reducing, sulfide oxidizing bacteria (NR-SOB) proliferate when nitrate is added to anaerobic wastewater containing sulfide (De Gusseme et al., 2009; Garcia-de-Lomas et al., 2007). If this process occurs in sewer conditions, it could play a significant role in sulfide control.
3. What is the impact of nitrate addition on methane concentrations in sewers? Substantial methane production has been recently reported in rising main sewers (Guisasola et al., 2008). Methane is a potent greenhouse gas (IPCC, 2006) and is explosive in confined spaces (Spencer et al., 2006). Methanogens, like SRB, thrive under anaerobic conditions, and their activity could potentially be inhibited by nitrate. The addition of nitrate for sulfide control may therefore have an additional beneficial environmental effect in controlling methane discharge from sewers.

Two laboratory scale sewer systems mimicking rising mains were used in this study. Both systems were fed with real sewage, with the experimental line also receiving nitrate. The research aims stated above were addressed by comparing the long-term performance of the two lines, measuring the activities of sewer biofilm in batch tests under various conditions, measuring the microscale distribution of sulfide and nitrate within the biofilm in conjunction with select batch tests, and by analyzing microbial composition using denaturing gradient gel electrophoresis (DGGE). Knowledge was gained through the integrated use of all information obtained.

2. Materials and methods

2.1. *Laboratory reactor experimental set-up and operation*

Two parallel laboratory scale rising main sewer systems (Fig. 1a), each consisting of four reactors (named RM1–4) connected in series, were operated in a temperature controlled laboratory (20 ± 1 °C). One system was used as the experimental line, while the other was maintained as a reference line. The inner diameter and volume of each cylindrical reactor was 80 mm and 0.75 L respectively. Biofilm was grown on the walls of the reactors, and on plastic Kaldnes carriers (circular, 1 cm diameter; Anox Kaldnes, Norway) placed on rods inside the reactors (Fig. 1b). The carriers facilitated intact biofilm removal for microscale and molecular studies. Further details of the reactor design and properties are available in Gutierrez et al. (2008). Mixing in the reactor was provided by the pump flow and by the magnetic stirrer, the contribution of the latter being dominant. The stirring conditions in the reactor corresponded to a Reynolds number of 6400 indicating a turbulent flow regime.

Both lines were fed intermittently with real sewage through a peristaltic pump (Masterflex Model 7520-47). Operation of the pump was programmed to mimic that of the UC09 rising main, which is a 1.1 km rising main located at the Gold Coast,