Optimizing Denitrification Efficiencies at Low-Temperature Conditions for the Enhancement of Site-Specific Bioremediations Solutions.

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Abstract

The effect temperatures have on denitrification efficiencies was evaluated for adapted indigenous bacterial communities' use as a bioremediation approach for contaminated mining sites located in low-temperature environments. Utilizing a kinetic exponential decay relation, denitrification rates were characterised using optimal carbon electron donor augmentation, derived from a carbon-molar ratio calculation. The kinetic data was applied in an adapted two-phase analytical decay model to predict the hydraulic retention times (HRT) at various temperature ranges. Denitrification efficiencies and HRT predictions were compared to high COD/N environments, using fixed filmed bioreactor treatment plants that are operated at low COD/N ratio, elevated nitrate (235 mg/L) levels, and temperatures ranging from 6 ^{0}C to 25 ^{0}C. Nitrate removal efficiency varied between 99% and 65% for this temperature range, while using a 20% lower carbon augmentation concentration than commonly described. On-site operational data suggest that denitrification rates in low COD/N environments are less sensitive under 10 °C, compared to in high COD/N environments. Additionally, the obtained denitrification efficiency was achieved at faster HRTs than analytically predicted, proposing that temperature is not the only rate-affecting factor to consider when designing and operating large-scale treatment plants for low-temperature conditions.

Keywords: Denitrification, fixed-film bioreactors, exponential decay, nitrate, and temperature.

Introduction

Nitrogen is one of the most abundant elements found on earth, while nitrate (NO_3) is one of the most common nitrogen-based compounds that occur naturally in moderate concentrations. Since the 1970s, nitrate contamination of ground – and surface water has become a significant environmental problem. The consequences include long-term health concerns arising from increased risks of methemoglobinemia, cancer, and environmental impacts such as the eutrophication of surface waters due to excess nutrients (Powlson *et al.* 2008). The sources include agricultural activities and sewage ingress.

Mining activities can also be regarded as a liberator of elemental nitrogen (occurring in the soil or geological formations) as a result of disturbance of bedrock through blasting, resulting in the release of nitrogen-based compounds. These compounds interact variable with water and dissolved oxygen. Since mining operations demand a high

volume of water usage during operations and tailing deposits, this aggravates a mobilisation problem of nitrate-impacted water. This impacted water has a detrimental impact on the environment and end receptors

In recent years the extended use of bioremediation treatments at mining sites has created a higher demand for effective and optimal biological denitrification approaches (Kuyucak 1998). Most bacteria responsible for denitrification are recognised as facultative heterotrophs and utilise nitrate as an electron-acceptor transforming it into nitrogen gas (Carrera *et al.* 2003). A better understanding of integrated metabolic cycles and how to manage denitrification efficiencies with electron-donor selection (carbon sources), augmentation concentrations, supplementation requirements, hydraulic retention times (HRT), and redox potential can identify the most effective remediation solution in a specific environment with an indigenous adapted bacterial community (Lau *et al.* 2016).

Considering that mining water contains low COD/N ratios, it is essential to augment the remediation process with an external carbon source, such as ethanol, acetate, or fatty acids (Bilanovic *et al.* 1999). Most wastewater systems utilize a COD/N ratio of 10:1 to sustain denitrification rates, while adapted indigenous bacterial communities from low COD/N environments, such as mining water, can effectively sustain denitrification activity at a 3:1 ratio (Hoffmann *et al.* 2007). Several publications report a preference for a specific carbon source while the variation emphasises the importance of characterising the optimum source for every site's indigenous bacteria, and its impact on denitrification rates, especially for continued larger-scale treatment plants.

Denitrification rates can be described in a typical exponential decay curve shown in equation 1 (Appelboom *et al.* 1990),

$$[C_1] = [C_0] \times e^{(-k \times t)} \quad (1),$$

where $[C_0]$ is the initial nitrate concentration, $[C_1]$ is the concentration at time *t*, and *k* is the decay coefficient. This equation is expressed as an overall average in units of mass over time but is limited since there is no reference to the initial nitrate concentration or temperature and its effects on denitrification rates. The Arrhenius-type equation 2 is used to express the effect of temperature on denitrification rates:

$$r_{D,T1} = r_{D,T2}^{\theta(T1-T2)} \quad (2),$$

where $r_{D,T1}$ is the denitrification rate at temperature T_1 and $r_{D,T2}$ at T_2, while θ equals the temperature coefficient. These aspects are merged in this paper to generate an adapted model, by considering removal efficiency, influent nitrate concentrations, and carbon stoichiometry at variable temperatures as a mass removal per volume over time. This allows the correlation of higher concentrations of nitrate removal efficiencies to effective HRTs for each temperature, especially at temperatures below 15 °C.

Denitrification efficacies obtained from fixed film bioreactors, operated at sites with elevated nitrate contamination (235 mg/L) and lower temperature environments, were compared to rates described from high COD/N wastewater treatments. Understanding the integration of denitrification efficiencies and variable environmental conditions can hold the key to developing more effective and sustainable biological treatment systems. This will impact design proficiencies and ultimately capital expenditure for larger-scale treatment plants, while showcasing lower operational costs associated with effective turnover, making bioremediation treatment systems even more accessible (Szablowski 2002).

Methods

Mining sites selection

Two sites with different treatment approaches were evaluated:

Site 1: Single-stage cylindric bioreactor treatment plant

A treatment plant was designed and operated, by the authors of this paper, at a mine in the Lesotho highland mountains. The surface water had a nitrate range of 210 – 235 mg/L, while temperatures varied between 6 °C and 25 °C. As such, iWater's biological plant was equipped with two proprietary designed fixed film bioreactors to evaluate denitrification rates at these temperature ranges. Bioreactor 1 was not temperature controlled and operated between 6 °C and 20 °C, while bioreactors

2 was constantly controlled at temperatures between 20 – 25 °C. Inlet site water was continuously augmented with sodium acetate as a carbon source. Each bioreactor's carbon concentrations were managed according to the HRT, which varied between 12 – 96 h at temperatures of 25 – 6 °C, respectively. All operations, in terms of carbon concentrations, HRT, redox conditions, and pH levels were managed remotely with a Program Logic Controller (PLC).

Site 2: Three-stage bioreactor treatment plant (USA-based)

This plant, compare to site 1, mainly operated at similar influx nitrate concentrations and temperatures, while a different carbon source selection and concentrations were augmented with extended HRTs. Site 2 is located in Montana, United States of America (USA), and consists of a three-stage bioreactor system. The bioreactors were operated in series with a single initial carbon augmentation with ethanol. Each bioreactor housed granular activated carbon (GAC) for a bacterial attachment media with low porosity. The bioreactors were not temperature controlled and operated between 6 – 20 °C. To achieve maximum denitrification rates HRT varied between 19 h, 34 h, 42 h, 53 h, and 56 h, for temperature ranges of 20 °C, 15 °C, 10 °C, 8 °C, and 6 °C, respectively.

Carbon-molar calculations and augmentation concentrations

For site 1, the required carbon concentration was characterised by applying a carbon-molar ratio, derived from a balanced carbon-base reaction (table 1) to the site's nitrate concentration, including a 10% sulfate level, for effective nitrate removal. The carbon source was also supplemented with Di-Potassium Hydro-orthophosphate (30:1).

Temperature vs HRT analytical model

To express denitrification rates under various environmental conditions a kinetic exponential decay (k) was calculated for nitrate removal efficiencies. To calculate k at different temperatures equation 1 (Appelboom *et al.* 2006) was expressed as a linear relation by calculating the natural log of both sides, as per equation 3:

$$\ln[C1] = -k \times t + \ln[C0] \quad (3),$$

where k is the slope of a straight-line relationship relating fixed time (t) and 99% removal efficiency (ln[C0] – ln[C1]) to variable volumes and temperatures (25 °C, 20 °C, 15 °C, 10 °C, 8 °C, and 6 °C), with ln[C1] being the y-intercept.

The effect of temperature on the denitrification efficiency is correlated to HRT, as a function of nitrate removal per volume. To express the representation between temperature and HRT, k is converted to a two-phase exponential decay ($r^2 = 0.99$) using equation 4 (Arango-Osorio *et al.* 2003):

$$HRT = \frac{1}{k} \times \left(\frac{C0 - C1}{C0}\right) \quad (4),$$

Where C_0 is the influx nitrate concentration at time t, and C_1 is the nitrate removed concentration. During operations, donor supplementation and availability were unchanged, while pH remained between 7.5 – 8.8.

Hydrochemistry analysis methods

All inorganic chemical and physicochemical analyses were conducted at South African National Analytical Standards (SANAS) accredited laboratories, using anion analysers, ICP-MS/OES, and auto-titration methods.

Results and Discussions

Treatment implementations are mainly focused to create an optimal environment for the indigenous bacteria to stimulate

Table 1 *Calculated carbon-molar ratios of stoichiometric electron donor demand for redox reactions of anaerobic metabolic electron acceptors.*

Electron acceptor	Balanced reaction	Molar ratio
	Acetate	
Oxygen	$CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O$	
Nitrate	$5CH_3COOH + 8NO_3^- + 8H^+ \rightarrow 10CO_2 + 4N_2 + 14H_2O$	
Sulfate	$CH_3COOH + SO42^- + 2H^+ \rightarrow 2CO_2 + H_2S + 2H_2O$	

selected community members to reduce site pollutants, as described in section 2.1. On-site operational and chemical data were used to generate an analytical model and compared to literature data sets composed from high COD/N ratio environments, such as wastewater treatments.

Carbon source selection and augmentation concentrations

It is essential to characterise an indigenous bacterial community's optimum carbon source and demand requirements using a microcosm study (Lau *et al.* 2016). The carbon-molar ratio calculator was utilized to characterise the minimum carbon concentrations, for site 1, to supply enough energy to sustain metabolic functioning at anaerobic redox conditions. This particular indigenous bacterial community is more selective toward a simple carbon structure source (e.g., sodium acetate), with minimum phosphate supplementation required.

To showcase the efficiency of the calculator, carbon usage is expressed as a value of COD/N ratio consumed:

$$\frac{COD}{N} = \frac{[COD]0-[COD]1}{C0-C1} \quad (5),$$

Where [COD]0 is the initial COD and [COD]1 is after treatment. The average value was 2.4 mg COD mg/N. The average stoichiometric ratio proposed for denitrification processes is 3 mg COD mg/N (EPA 1993; Mateju *et al.* 1992). The carbon-molar ratio calculator offers a 20% better yield efficiency in low COD environments. When it is considered for large-scale treatments (1 meqL/day) the utilisation of donor requirements per year can be considerably less.

Denitrification rate and temperature

The effect of the surrounding environmental temperatures on the denitrification rate is essential when designing a biological treatment plant. These factors can affect operational parameters, such as carbon concentrations and HRTs, which must be correlated to innovative engineering to either ensure variable operational parameters availability or temperature controlling affects. These considerations can have a major cost-saving capability for large-scale treatment plants.

The effect that temperature has on denitrification rates was considered together with removal efficiency and influx nitrate concentrations, using equations 3 and 4 (assuming donor stoichiometry remains unchanged with a constant pH of 7.5 – 8.8). Note that sites 1 and 2 (low COD) were compared to literature data (high COD) (Carrera *et al.* 2003; Doyle *et al.* 2001; and Oleszkiewicz *et al.* 1988). Figure 1 shows that the predicted HRT for denitrification is 19 times higher at 6 °C compared to 25 °C at high COD availability. In comparison to only 9 times for the same temperatures at low COD environments. Additionally, the denitrification rate has a higher sensitivity at temperatures below 10 °C, independent of the donor source or availability (fig. 1). These are important factors to consider since different design parameters and operating approaches must be implemented in different COD availability environments. Denitrification rate sensitivity to temperatures below 10 °C has been described previously (Carrera *et al.* 2003; Dawson and Murphy 1972; and Kim *et al.* 2006), however, this data indicates a less sensitive effect than commonly suggested and that faster HRTs can be implemented to still achieve effective nitrate removal efficiencies.

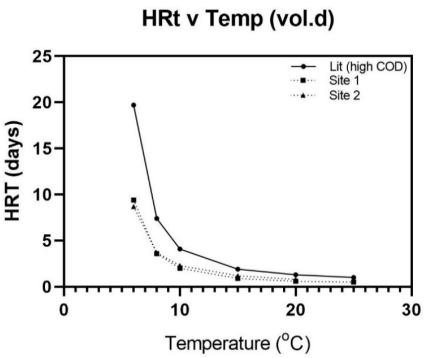

Figure 1 *Effect of temperature on the HRT.*

Site operations and associated denitrifying efficiencies

Following the predicted HRT control, site 1 was operated accordingly. Through HRT adjustments between 12 h – 96 h, the plant was operated to achieve rate determining nitrate removal at temperatures ranging from 6 – 25 °C, respectively (table 2).

Table 2 Treated chemical data set of site 1 at various temperatures and HRT.

		Site 1					
Temperature (°C)		25	20	15	10	8	6
HRT (h)		12	12	24	48	72	96
Parameter	Unit	Inlet	Outlet				
Alkalinity - total	mg $CaCO_3$/L	54	449	445	447	307	98	95,1
pH		7,5	8,8	8,4	8	7,6	7,6	7,6
Nitrate	mg/L	234	< 0,01	0,5	5,7	18,8	79,8	70,5
Nitrite	mg/L	< 0,01	< 0,01	0,60	10,6	19,5	-	-

Interestingly, the model is on par at temperatures above 15 °C, however, the bioreactors were operated at slightly faster HRTs below 15 °C, while achieving a 65% nitrate removal efficiency (figure 2 and table 2). A wide range of literature advises that up to 60% and 90% of nitrate removal efficiency are lost at temperatures below 15 °C and 8 °C, respectively (Qu *et. al.,* 2022; Saleh-Lakha *et al.,* 2009; Vacková *et al.,* 2011). Qu and co-workers' future described carbon utilization by the bacteria also significantly reduce at temperatures below 15 °C, independent of the carbon source used. The nitrate removal efficiency at these low temperatures is rarely seen and achieving it at such fast HRT and a constant 2.4 mg COD mg/N augmentation, makes it even more unique.

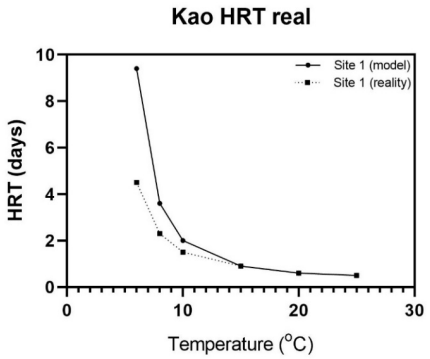

Figure 2 Theoretical temperature effect on HRT compared to actual operational HRT.

To clarify the analytical model prediction at lower temperatures one can, attribute these rates to more factors than just temperature, donor availability and pH. Metabolic function and adaptability within the indigenous bacterial community can also contribute to denitrification rates. It is well described that partial denitrification metabolism frequently occurs in environments with low DOC/N ratios and at temperatures below 15 °C (Almeida *et al.,* 1995; Ge *et al.,* 2012; Wilderer *et al.,* 1987). This has also been observed during site 1 operations, where up to 19 mg/L nitrite (NO2-) was present for a 165 mg/L nitrate removal efficiency (table 2). This indicates a slight stall in metabolic activity as temperatures lower. This can have a major effect on denitrification efficiencies, as accumulative levels of nitrite can have a feedback inhibition for denitrifying bacteria (Almeida *et al.,* 1995; Carrera *et al.* 2003). Extended metagenomic studies can determine whether all the required denitrifying genes are present or expressed, or does it include alternative functionality to allow denitrification to continue throughout these temperature ranges as a new conundrum. By future comparing the metagenomic data between different treatment implementations, the often-excluded bacterial diversity and functionality aspect could answer this question.

Conclusion

This paper highlights the need to determine the optimum carbon source per indigenous bacterial community, especially for low COD/N environments. When carbon sources are stoichiometrically balanced to the geohydrochemical nitrate conditions, up to 20% lower carbon augmentation can be used, which has major operational cost-saving implications for large-scale biological treatment plants. The adapted analytical exponential decay model indicates that denitrification efficiencies are highly sensitive at lower temperatures, nevertheless, adapted indigenous bacteria from low COD/N environments are prone to be less affected. This consideration is essential when designing

and operating denitrifying treatment plants in mining environments. When this analytical exponential decay model and predicted HRT requirements were implemented for a fixed film bioreactor plant, 99% nitrate removal was achieved at temperatures above 20 °C, showcasing a fairly accurate prediction. Nevertheless, at lower temperatures slightly faster HRTs, than predicted, were achieved, with a 70% nitrate removal efficiency at 6 °C. This removal efficiency is rarely described at such low temperatures. It is, however, important to note that a partial denitrification metabolic stall at nitrite was analysed, suggesting that metabolic functioning and adaptability also play a role in denitrification efficiencies at lower temperatures. Thus, it is essential to identify all the informational gaps of each site to tailor the remediation strategy since no aspect from the geohydrochemistry, environmental conditions, carbon source selection, bacterial composition, and metabolic capacities stand alone.

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