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Effect of temperature & salt concentration on salt tolerant nitrate-perchlorate reducing bacteria: Nitrate degradation kinetics

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ABSTRACT

The sustainability of nitrate-contaminated water treatment using ion-exchange processes can be achieved by regenerating the exhausted resin several times. Our previous study shows that the use of multi-cycle bioregeneration of resin enclosed in membrane is an effective and innovative regeneration method. In this research, the effects of two independent factors (temperature and salt concentration) on the biological denitrification rate were studied. The results of this research along with the experimental results of the previous study on the effect of the same factors on nitrate desorption rate from the resin allow the optimization of the bioregeneration process.

The results of nitrate denitrification rate study show that the biodegradation rate at different temperature and salt concentration is independent of the initial nitrate concentration. At each specific salt concentration, the nitrate removal rate increased with increasing temperature with the average value of 0.001110 ± 0.0000647 mg-nitrate/mg-VSS.h.°C. However, the effect of different salt concentrations was dependent on the temperature; there is a significant interaction between salt concentration and temperature; within each group of temperatures, the nitrate degradation rate decreased with increasing the salt concentration. The temperature affected the tolerance to salinity and culture was less tolerant to high concentration of salt at low temperature. Evidenced by the difference between the minimum and maximum nitrate degradation rate being greater at lower temperature. At 35 °C, a 32% reduction in the nitrate degradation rate was observed while at 12 °C this reduction was 69%. This is the first published study to examine the interaction of salt concentration and temperature during biological denitrification.

1. Introduction

Contaminant release from industrial and agricultural activities into water supplies is increasingly affecting the water quality, resulting in a public health crisis (Islam and Patel, 2010; Campbell et al., 2006; Romano and Zeng, 2009). Nitrate contamination of water is one of the largest issues facing communities in different parts of the world, especially areas with high agricultural activities.

Nitrate contamination creates many environmental and health issues (Romano and Zeng, 2009; Hudak, 2000; Hall et al., 2001). To limit the risk to human health from nitrate in drinking water, in North America the maximum acceptable concentration of nitrate in drinking water is regulated to be less than 10 mg/L as nitrogen or 45 mg/L as nitrate (USEPA, 2010).

Different technologies can be used to remove nitrate from water, and ion exchange (IX) is one of the most commonly used methods (Ginner et al., 2004; Biswas and Bose, 2005; Bae et al., 2002). Water treatment utilities prefer to use nitrate highly selective resins because of their great capacity; they can be used for 100,000 to 200,000 bed volumes. The most challenging issue with using these selective resins is the lack of viable methods for regenerating them. Selective resins can be regenerated using very high concentrations of salt (≈12%) and a large amount of regeneration solution or using chemicals such as ferric chloride, hydrochloric acid, tera-chloroferrate, etc. which is not economically feasible, thus rendering them as single use resins (Aldridge et al., 2005; Aldex Chemical Company, 2015; DeSilva, 2003; Brown et al., 2002). At the industrial level, at this point, single use exhausted resins are replaced with fresh resin and the exhausted resin is disposed of to the landfill or incinerated. In addition to the high cost of resin production, and shipping to the landfill and incineration sites, the environmental effects of these methods are undeniable. So, because

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of the wide use of ion exchange processes as a promising method for nitrate removal from contaminated water, developing methods for regeneration of exhausted single use resins is urgent. Increased sustainability of nitrate-contaminated water treatment processes can be achieved by regenerating the exhausted resin several times which is the main goal of our research group.

There have been a few publications describing the development of methods for treatment and regeneration of the produced brine from the regeneration flow from resins that are selective for nitrate but are still regenerable with a 3–6% salt brine (Yang et al., 2013; Li et al., 2015; Bae et al., 2002, 2004; McAdam and Judd, 2008; Lehman et al., 2008). Direct bioregeneration of perchlorate-selective resin has also been studied by a few researchers (Venkatesan et al., 2010; Xiao et al., 2010). Since in this process, the resin is in direct contact with the culture, at the end of the each regeneration cycle, extra processes including bio-fouling removal and resin disinfection are required before using the resin in another ion exchange cycle. Recently, Meng et al. (2014) reported the bioregeneration of exhausted resin for nitrate treatment from water. Although they have looked at the effect of different environmental parameters on the regeneration, they have not mentioned the effect of the bioregeneration on reuse of the resin or any post-processing of the resin before using the resin in another ion exchange cycle. In all of these studies, because of the direct contact of biomass with resin, more than the extra inevitable processes, there is a possibility that the biomass will affect the desorption of the perchlorate/nitrate from the resin. These factors result in increasing the cost and time required for each regeneration cycle (Venkatesan et al., 2010).

To improve this sustainable method for nitrate removal from water, we have enclosed the resin in a membrane to prevent direct contact of the resin with the bacterial culture during the bioregeneration stage. In the first step of our research, as a proof of concept, the use of multi-cycle exhaustion/bioregeneration of resin enclosed in membrane was shown to be quite effective; the salt tolerant culture was capable of regenerating the resin and allowing it to be used for 4 cycles of exhaustion without losing capacity, and only 6% capacity lost after 6 cycles (Ebrahimi and Roberts, 2013).

In order to model and design a commercial scale process, an understanding of the major environmental operational parameters for resin regeneration is necessary. Bioregeneration of resin includes desorption of nitrate from resin and degradation of nitrate in the aqueous phase at the same time. Therefore, the effect of environmental parameters should be investigated on these two processes separately to provide enough information to model overall bioregeneration of the resin. Current presented study focuses on the second process, biological removal of nitrate in the aqueous phase.

Removal of nitrate from the aqueous phase can occur through different pathways including, denitrification which is converting nitrate to nitrogen gas, and dissimilatory nitrate reduction to ammonia (DNRA). Complete denitrification and prevention of DNRA can be controlled by providing excess carbon source for the biomass (Megonigal et al., 2004; Metcalf and Eddy, 2004).

There are several studies looking at the effect of temperature and salt concentration on the biological denitrification rate as separate factors (Chung et al., 2007; Dawson and Murphy, 1972; Elefsiniotis and Li, 2006; Ucisik and Henze, 2004; Glass and Silverstein, 1999; Van der Hoek et al., 1987; Lewandoswki, 1982). But to the best of our knowledge there is no study on the interaction of salt concentration and temperature.

Higher concentrations of salt and higher temperature are beneficial for regeneration of the resin since the initial desorption rate of nitrate from resin is increased by increasing the temperature and salt concentration (Ebrahimi and Roberts, 2015). On the other hand, high temperature may improve nitrate degradation by the culture to a certain extent, but high concentrations of salt can have negative impact on biomass performance. Since in direct resin bioregeneration the desorption of nitrate from resin and degradation of nitrate is happening at the same time, it is important to find the best environmental condition for both desorption and degradation processes. In this research, the effect of two independent factors (temperature and salt concentration) on denitrification rate (as dependent parameter) were studied to allow the optimization of the bioregeneration process along with the previous study of the effect of same factors on the nitrate desorption rate from the resin (Ebrahimi and Roberts, 2015).

The main objectives of this study are to understand and investigate 1) the effect of salt concentration on the nitrate removal rate considering 4 different levels of salt, 2) the effect of temperature on denitrification including 3 different temperatures, 3) the interaction of temperature and salt on the nitrate removal rate, and, 4) to mathematically describe the effect of temperature and salt concentration on nitrate removal rate for future use in a bioregeneration model.

2. Materials & methods

2.1. Culture, medium and bioreactor

A mixed perchlorate- and nitrate-reducing bacterial culture (NP30) was enriched from marine sediment and studied well in previous research (Cang et al., 2004; Ebrahimi et al., 2012; Stepanov et al., 2014; Xiao and Roberts, 2013). A derivative of NP30, named N30 was kept at 3% salt, fed nitrate at room temperature using synthetic medium with the addition of a group of trace metals. The medium contained, 1.4 g/L $CaCl_2 \cdot 2H_2O$, 0.72 g/L KCl, 0.3 g/L $NaHCO_3$, 0.48 g/L NH_4Cl, 1 mL/L 67 mM $Na_2S \cdot 9H_2O$, 1 mL/L phosphate solution (50 g/L KH_2PO_4), 1 mL/L mineral solution (which contained 50 g/L $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.05 g/L $ZnCl_2$, 0.3 g/L H_3BO_3, 1.5 g/L $FeCl_2 \cdot 4H_2O$, 10 g/L $CoCl_2 \cdot 6H_2O$, 0.03 g/L $MnCl_2 \cdot 6H_2O$, and 0.03 g/L $NiCl_2 \cdot 6H_2O$). In this research we controlled the salt concentration with NaCl at different concentrations; 20, 30, 40, 50, and 60 g/L for 2, 3, 4, 5, and 6% salt, respectively. Also, the ratio of the Mg/Na was kept at 0.11 mol/mol (using $MgCl_2 \cdot 6H_2O$) as it is required for the stable activity of the culture in high concentration solutions (Xiao and Roberts, 2013; Lin et al., 2007). Also, nitrate and acetate concentrations were controlled using $NaNO_3$ and $CH_3COONa \cdot 3H_2O$ to get 100 and 500 mg/L nitrate wherever was desired and to achieve 1.5 acetate to nitrate ratio as the carbon and electron source. Strict anaerobic technique was used in medium preparation by boiling the medium and nitrogen gas flush while transferring.

Monitoring the nitrite in the system showed that there is no nitrite accumulation in the system. A nitrogen mass balance and gas production of the system showed that the complete denitrification of nitrate to the nitrogen gas had occurred in all cultures.

2.2. Culture adaptation in batch bioreactors

Salt concentration and temperature were considered as two independent factors and the nitrate degradation rate as the dependent factor. Using factorial design of experiment method, a series of batch reactors were designed to reveal the effect of temperature and salt concentration on the nitrate degradation rate. Five levels of salt concentration (2, 3, 4, 5, and 6% salt) and three temperatures (10, 23 and 35 °C) were examined. Early studies showed the culture was sensitive to rapid changes in salt or temperature and so a two phase adaptation method was used; salt-temperature and temperature-salt adaptation methods. In the