Chapter 1

Introductory Chapter: Effects of Salinity on Biological Nitrate Removal from Industrial Wastewater

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1. Introduction

Nitrification and denitrification is a major process route for nitrogen cycles in ecological systems. It is essential for nitrogen removal from water and wastewater. In the past, there were tremendous efforts and a significant amount of research on this topic in regard to microbial species, effects of operating parameters such as temperature, pH, dissolved oxygen (DO), types of carbon sources, and hydraulic and mass loadings. In this book, a comprehensive review of conventional technologies was carried out and innovative technologies such as *anaerobic ammonium oxidation* (*Anammox*) were focused upon. A unique species *Alcaligenes faecalis* No. 4 was reviewed and experimented for heterotrophic nitrification and aerobic denitrification. Aerobic biofiltration for ammonia removal and an anoxic membrane bioreactor (MBR) for nitrate removal were selected as application examples.

However, the effect of salinity on nitrification and denitrification was not discussed. Some industrial wastewater contains high concentrations of salinity and nitrate, that is, flue-gas desulfurization (FGD) wastewater contains nitrate (about 50 mg/L as N), high total dissolved solids (TDSs) (1–5% of chloride), and other contaminants. Elevated TDS levels present in industrial wastewater may have profound effect on denitrification and there had been little research on this subject matter. To remove nitrate from such wastewater, it is important to understand the effects of salinity on the process kinetics, selection of carbon sources, periodical salinity fluctuations, and microbial communities for the process selection and engineering design.

2. Selection of carbon sources in process design

Heterotrophic denitrification occurs in the presence of both nitrate and biodegradable organic substances under anoxic conditions. If a denitrification system is placed after a secondary wastewater treatment process, intrinsic biodegradable organic substances are essentially

Carbon source	COD/N	Yield (gVSS/gCOD)	μ_{max} (d^{-1})	k_D (mgN/gVSS-h)
Methanol	4.1–4.5	0.23–0.25	0.77 (15°C) 2 (20°C)	32 (15°C) 91 (20°C)
Ethanol	5.9	0.25–0.28	1.89 (15°C) 4.8 (25°C)	46 (15°C) 139 (20°C)
Acetate	5.7	0.35	1.2 (13°C) 3.5 (19°C)	13.6
Glucose	8.9	0.38		3.8

Table 1. Kinetic information of selected carbon sources [1].

depleted before the denitrification unit. Under these situations, external supplementation of organic substances (electron donors) is usually needed to generate dedicated microbial communities. Generally, an external carbon source, such as methanol, ethanol, acetic acid, glycerol, sugar, or molasses, is used as a supplement. Another commercial product worth mentioning is MicroC™. It is manufactured by Environmental Operating Solutions Inc. (Bourne, MA) and is an environmentally benign, proprietary wastewater treatment chemical containing a mixture of organic compounds, mainly glycerol. It contains 670,000 mg/L chemical oxygen demand (COD) with a specific gravity of 1.22 g/mL at 25°C.

The stoichiometric reaction C/N ratio, yield, specific growth rate, and Arrhenius temperature factor are different for different carbon sources, some of which are summarized in **Table 1**. It should be advised that these parameters were obtained with municipal wastewater under different acclimation and feeding conditions and microbial compositions, and the use of these parameters should be with care.

3. Impact of salinity on specific denitrification rate

Specific denitrification rates (SDNRs) are usually expressed in the mass of nitrate removed within a unit time in regard to one unit of reactor volume, biomass, biofilm surface, or fixed-film media bed. There had been conflicting reports about the effects of salinity on specific denitrification rates.

Osaka et al. [2] studied two suspended biomass systems fed with acetate acid and methanol, respectively, and found that acetate-fed process attained high nitrate removal at 0–10% NaCl, whereas methanol was shown effective for nitrate removal at 0–3% NaCl without sacrificing efficiencies. Nitrate removal efficiencies were close to 100% at a mass loading of 0.15 g NO_3-N/g MLSS/day or a volumetric loading of 0.75 kg NO_3-N/m^3/day. This study was carried out in a manner that allowed enough time (at least 20 days) for microbial communities to adapt to a higher salinity with a 1% incremental change.

Similar to the observation by Osaka et al. [2], the denitrification rate with methanol as a carbon source was unaffected by sodium chloride up to 2% in a fluidized bed biofilm reactor with media carriers encapsulated with mixed denitrification cultures [3].

SDNR was 0.06 g NO_3-N/g MLSS/day for a freshwater system without salt spiking; SDNR appeared not to be affected (similar to 0.06 g NO_3-N/g MLSS/day) for a system with 5 g/L salt

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spiking, and it only slightly decreased to 0.048 g NO_3-N/g MLSS/day for an acclimated system with 30 g/L salt addition [4]. In fully acclimated systems (two bench-scale sequencing batch reactors operated in parallel for 4 months), as complete denitrification occurred, the maximum specific nitrate reduction rate was 1.2 g NO_3 ± N/g MLSS/day at a wastewater TDS concentration of 4.8% with acetate as a carbon source and the denitrification rate was decreased to 0.456 g NO_3 ± N/g MLSS/day at 18% TDS [5]. These studies suggest that acclimated (to saline water) systems appeared less sensitive to salinity increase.

The maximum nitrification and denitrification rates were 0.05 and 0.036 g NO_3-N/g VSS/day, respectively, in a down-flow hanging sponge reactor treating phenol (electron donor for denitrification) and ammonia wastewater. The system had been acclimated for 1100 days with 10.9 g/L chloride before the study where a dominant species, *Azoarcus*-like species, was found [6]. The maximal denitrification rate achieved with ethanol mixture (industrial byproduct) (0.64 g N-NO_x/g VSS/d) was much higher than the rate reached with methanol mixture (industrial byproduct) (0.11 g N-NO_x/g VSS/d) at sulfate concentrations of 1.5–2% after 450 days of operation [7]. Pure culture *Pseudomonas stutzeri* in a packed bed bioreactor achieved high denitrification rate of 0.84 kg NO_3-N/(m^3/day) or 0.025–0.13 g NO_3-N/g biomass/day at 10 g/L salinity [8]. The strain PAD-2 (closely related to *M. alkaliphilus*) in genus *Marinobacter* of *γ-proteobacteria* exhibited higher denitrification rates at concentrations of 3–6% than at other salinities of 12–18% w/w [9].

On the contrary to the above studies, it was concluded that denitrification rates were severely affected with salt spiking. At 1.52% of salt spiking, a specific denitrification rate decreased by half from 0.7 to 0.35 kg NO_3-N/m^3/day [10]. In another study by Ucisik and Henze [11], it was found that a specific denitrification rate decreased with an increasing chloride concentration in a suspended growth system fed with acetate, and the maximal specific denitrification rate decreased from 1.2 kg NO_3-N/m^3/day at 0.48% chloride down to 0.04 kg NO_3-N/m^3/day at 9.67% chloride. However, this study may still have suffered from insufficient acclimation time, as at each chloride concentration level, the microorganisms were only allowed to acclimatize for 4–5 days. The spiking of salt sharply reduced the microbial activity in an activated sludge system seeded with municipal sludge. When salt concentrations were below 10 g/L NaCl, microorganisms were able to acclimatize in several weeks and achieve the same initial activity as in raw sludge samples; when the salt concentration was above 30 g/L NaCl, the acclimatization process was slow [12]. A mathematical model was developed to predict the SDNR at different salt spiking levels where a salt inhibition constant was identified to be 1.52% (SDNR was reduced by half) [10].

Table 2 summarizes SDNR in high-salinity wastewater and SDNR varied from 0.75 to 4.8 kg NO_3-N/m^3/day or 0.025 to 1.2 g/g biomass/day, depending on the salinity levels, carbon sources, and temperature. It appeared that biofilm systems had relatively higher volumetric denitrification rates as compared to the suspended growth systems. A maximal denitrification rate of 4.8 kg NO_x-N/m^3 media bed/day(sintered fly ash) was achieved in a fluidized bed reactor; 2.5 kg NO_3-N/m^3/day was achieved with a reactor filled with sponge cubes for microbial attachment; and 0.84 kg NO_3-N/m^3/day was achieved in a packed bed reactor (with clinoptilolite). These observations of high rates were perhaps attributed to higher specific surface area of carrier media and higher biomass density. Furthermore, in a biofilm reactor filled with cellulose triacetate carriers encapsulated with mixed denitrification cultures, an exceptionally high denitrification rate of 11 kg/m^3 media bed/day was achieved [3].

Denitrification rate	Acclimation and culture	Carbon source	Salinity	System	Reference
0.84 kg NO_3-N/(m^3/day) at 10 g/L salinity; 0.025–0.13 g NO_3-N/(g biomass.day)	*Pseudomonas stutzeri*	Ethanol	10–40 g/L	Packaged bed system (clinoptilolite)	[8]
0.75 kg NO_3-N/m^3/day or 0.15 g NO_3-N/g MLSS/day (10% salinity with acetic acid)	The saline concentration was steadily increased by 1% salinity with NaCl from 0%; at each salinity level, at least 20 days were maintained	Acetate and methanol	0–100 g/L	Suspended growth system	[2]
1.2 kg NO_3-N/m^3/day at 4.8 g/L chloride; 0.04 kg NO_3-N/m^3/day at 96.7 mg/L chloride	At each chloride level, 4–5 days were allowed for acclimation	Acetate	4.8–96.7 g/L	Suspended growth system	[11]
0.7 kg NO_3-N/m^3/day for 0% NaCl and 0.35 for 1.52% NaCl	Spiking	Sugar	0–6%	Packaged bed system (1 cm plastic tubes)	[10]
A slight drop in nitrogen removal, NR, and DNR was observed, when the salinity was increased from 4.2 to 9.8 g NaCl/L		Intrinsic COD	4.2–9.8 g NaCl/L	Sequential batch biofilm reactor	[17]
2.5 kg NO_3-N/m^3/day at 10% salinity	*Halomonas* sp. and *Marinobacter* sp.; seed sludge was acclimated for 3 years	Acetate	2 and 10%	Sponge cubic media	[14]
0.8 kg NO_3-N/m^3/day	*P. pantotrophus and P. fluorescens*	Biodegradable hydrocarbons Brenntaplus VP1	Up to 35 g/L Cl^- and 17 g/L SO_4^{2-}	Bacteria encapsulated in porous polyvinyl alcohol lenses	[15]
0.036 g/g-VSS/day	*Azoarcus*-like species; acclimated for 1100 days prior to the study	Phenol	10.9 g Cl^-/L	Down-flow hanging sponge reactor	[6]
0.64 g N-NO_x/g VSS/d with ethanol; 0.11 g N-NO_x/g VSS/d with industrial waste methanol	The two-sludge plant was operated continuously for 450 days, using real, high-strength industrial wastewater	Industrial ethanol mix; industrial methanol mix	1.5–2.0% SO_4^{2-}	Suspended growth system	[7]
1.2 g NO_3 ± N/g MLSS/day at TDS 4.8%; 0.456 g NO_3 ± N/g MLSS/day at 18% TDS	Reactors operated in parallel for 4 months	Acetate	4.8, 16, and 18%	Suspended growth system	[5]
4.8 kg NO_x-N/m^3 media bed/day	Acclimated	Acetic acid	45 g/L Cl^-	Fluidized bed system	[18]
11 kg NO_x-N/m^3 media bed/day or 4.8 kg NO_x-N/m^3/day	Media carriers encapsulated with mixed culture	Methanol	0–30 g/L NaCl	Fluidized bed system	[3]

Denitrification rate	Acclimation and culture	Carbon source	Salinity	System	Reference
0.06 g NO_3-N/g MLSS/day without salt addition; 0.06 g NO_3-N/g MLSS/day for 5 g/L salt and 0.048 g NO_3-N/g MLSS/day for 30 g/L salt	Acclimated	Sucrose and acetic acid	30 g/L NaCl	Suspended growth system	[4]
0.305 (on acetate); 0.36 (on lactate), 0.39 (on glycerol), and 0.045 (on ethanol) g NO_3-N/g biomass/day	*Halomonas campisalis* sp. *Nov.*		12.5% NaCl	Suspended growth system	[16]

VSS, volatile suspended solids; MLSS, mixed liquor suspended solids; TDS, total dissolved solids; COD, chemical oxygen demand.

Table 2. Denitrification rates under different conditions.

In summary, it appeared that denitrification efficiency will drop upon an initial increase of salinity and can be sustained if biomass is properly acclimated and adapted to corresponding salinities, and rates were comparable to that at low-salinity concentrations. However, in full-scale installations, this effect may be pronounced during the initial period of commissioning, which usually required the designer to provide enough redundancy for the process, or seed the process with an acclimated culture obtained elsewhere to speed up the process.

4. Halophilic cultures

Halophilic bacteria are microorganisms that do not need sodium chloride to grow but can grow in high-salinity environments. Halophilic bacteria are classified into three groups according to their response to sodium chloride concentrations: (i) the slight halophiles (most rapid growth at 2–5% NaCl), (ii) the moderate halophiles (most rapid growth at 5–20% NaCl), and (iii) the extreme halophiles (most rapid growth at 20–30% NaCl) [13].

The phylogenetic analysis showed that the strains isolated from acclimated sludge (to saline water) had a high similarity to the genus *Alcaligenes* in β-*proteobacteria* and the genera *Vibrio, Pseudomonas, and Halomonas* in γ-*proteobacteria*. Genera *Halomonas and Marinobacter* in γ-*proteobacteria* were isolated [14]. α-*Proteobacteria* were also found [6]. *Azoarcus*-like species in β-*proteobacteria* was identified to conduct denitrification using phenol [6]. It was found that the dominant species shifted when salinity varied [14].

Researchers used microorganisms *P. fluorescens* and *P. pantotrophus* for denitrification in saline water [15]. *P. stutzeri* in the packed bed bioreactor achieved a high denitrification rate at 10 g/L salinity [8], and the strain PAD-2 (closely related to *M. alkaliphilus*) in genus *Marinobacter* of γ-*proteobacteria* also exhibited high denitrification rates at concentrations of 3–6% [9]. The species

M. aquaeolei sp. *nov*. was found to grow under anoxic conditions in the presence of nitrate on succinate, citrate, or acetate, but not on glucose. It was also interesting that *H. campisalis* sp. *nov*. grew on acetate, lactate, glycerol, and ethanol but not on methanol [16].

Table 3 summarizes the species capable of denitrifying under saline conditions. Most of the species were in the class of *γ-proteobacteria*. Species were found to even survive in a wide range of salinity as high as 23.4%.

5. Summary and future perspective

Extensive research was conducted in the past for denitrifiers in treating municipal wastewater which typically contains TDS less than 1000 mg/L. Whether the knowledge acquired in regard to the stoichiometry, kinetics, metabolic pathways, and microbial communities and characteristics is transferrable to halophilic counterparts is subject to further research.

Through the above short review, it was suggested that specific denitrification rates of mixed cultures decreased with an increasing salinity concentration. However, some specific species such as *H. campisalis* sp. *nov*. exhibited relatively high denitrification rates at 12.5% salinity with different carbon sources, similar to that of its freshwater counterparts. If biomass is properly acclimated and adapted to saline environments, the SDNR could be comparable to that at low-salinity concentrations.

Class	Genus	Species	Salinity range	Optimal salinity	Reference
γ-Proteobacteria	*Halomonas*	*campisalis* sp. *Nov*.	1.17–26.3 % (w/v)	8.8% (w/v)	[16]
	Halomonas	*daqingensis* sp. *nov*.	1.0–15.0% (w/v)	5–10% (w/v)	[19]
	Halomonas	*ventosae* sp. *nov*.	1.0–15.0 % (w/v)	8% (w/v)	[20]
	Halomonas	*chromatireducens* sp. *nov*.	0.585–23.4% (w/v)	2.9% (w/v)	[21]
	Halomonas	*desiderata* sp. *nov*.	0–18% (w/w)		[22]
	Marinobacter	*aquaeolei sp. nov*.	0–20% (w/w)	5% (w/w)	[23]
	Marinobacter	*PAD-2 (closely related to M. alkaliphilus)*	3–18% (w/w)	3–6% (w/v)	[9]
	Pseudomonas	*stutzeri*	1–4% (w/v)		[8]
	Pseudomonas	*pantotrophus and fluorescens*	Up to 3.5% (w/v) Cl^- and 1.7% (w/v) SO_4^{2-}		[15]
β-Proteobacteria	*Azoarcus*	*Azoarcus*-like species	1.09% (w/v) Cl^-		[6]

Table 3. Summary of halophilic denitrifying species.

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