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Effect of salinity on nitrification efficiency and structure of ammonia-oxidizing bacterial communities in a submerged fixed bed bioreactor

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HIGHLIGHTS

- The effect of salt (NaCl) on biological nitrogen removal was studied.
- Nitrification process is inhibited at high salt concentrations (⩾24.1 g NaCl/L).
- Ammonia oxidizing bacterial communities were studied by 454-pyrosequencing.
- Only 5 OTUs of 42 OTUs were found at all salinities tested.
- *amoA* sequences related to *Nitrosospira* disappeared at high salinity.

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ABSTRACT

The effect of salt (NaCl) on biological nitrogen removal and community structure of ammonia-oxidizing bacteria (AOB) was investigated in a submerged fixed bed bioreactor (SFBBR). Influent wastewater was supplemented with NaCl at 0 (control), 3.7, 24.1 and 44.1 g/L, and the rate of ammonia removal efficiency was measured by ion chromatography. The structure of the AOB community was profiled by 454-pyrosequencing, based on the amplification of partial ammonia-monooxygenase subunit A (*amoA*) genes. Salinity did not inhibit nitrification at 3.7 g/L, while ammonia oxidation activity significantly decreased and nitrite was consequently accumulated in the SFBBR when the salt concentration was ⩾24.1 g/L. The sequencing of *amoA* genes revealed that many of the OTUs found in the control experiment were still present at the full range of NaCl studied, while concentrations of 24.1 and 44.1 g of NaCl/L promoted the emergence of new OTUs phylogenetically related to AOB described in saline environments.

1. Introduction

Nitrogen, one of the main compounds in wastewater, causes serious environmental problems. Eutrophication, excessive growth of algae that causes the death of other organisms such as fishes, and oxygen depletion are some of the adverse environmental impacts associated with excess N [1]. Because of their negative effects, nitrogen compounds should be removed from the wastewater before it is discharged to the environment.

Nitrification is a key process in biological nitrogen removal. It occurs in two separate reactions, in the first one (phase I) ammonia-oxidizing bacteria (AOB) oxidize ammonia to nitrite and in the second one (phase II) nitrite is oxidized to nitrate by nitrite-oxidizing bacteria (NOB) [2]. Generally, nitrification is considered as the rate-limiting step of the overall biological wastewater treatment process due to the low growth rate of the organisms involved [3].

Salt is considered a common stress factor able to destabilise the microbial communities in wastewater treatment plants (WWTPs) [4]. It is well known that osmotic stress in wastewater reduces bacterial metabolic activities [5]. Nitrification is particularly susceptible to inhibition by salt [6,7], although AOB and NOB are thought to respond differently to changes of environmental conditions such as varying salinity. High sensitivity of AOB to increasing salt concen-

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Table 1
Operational parameters of experiments conducted in the submerged fixed bed reactor during the experiments E0, E3.7, E24.1 and E44.1. Average values marked with the same letter are not significantly different, according to the least significant difference (LSD), test ($p < 0.05$).

	E0	E3.7	E24.1	E44.1	LSD
Influent					
COD (mgO_2/l)	980.26 ± 233.05[a]	877.13 ± 129.82[a,b]	971.17 ± 209.74[a,b]	915.37 ± 160.25[b]	135.48
BOD_5 (mgO_2/l)	425 ± 97.83[a]	418 ± 72.46[a]	370 ± 78.04[a]	416 ± 65.05[a]	57.34
TSS (mg/l)	622.43 ± 398.95[a]	746.21 ± 207.54[a]	467.32 ± 423.31[a]	661 ± 298.43[a]	139.67
VSS (mg/l)	520.48 ± 153.24[a]	640.71 ± 165.12[a]	370.32 ± 83.65[a]	541.65 ± 94.03[a]	65.79
pH	7.55 ± 0.21[a]	8.20 ± 0.28[b]	7.42 ± 0.18[c]	7.23 ± 0.17[d]	0.16
Effluent					
COD (mgO_2/l)	78.47 ± 28.64[a]	89.50 ± 19.59[a]	312.76 ± 34.21[b]	553.14 ± 54.04[c]	23.87
BOD_5 (mgO_2/l)	17 ± 6.51[a]	163 ± 42.22[b]	247.33 ± 75.42[c]	358.66 ± 65.26[d]	48.12
TSS (mg/l)	19.48 ± 7.61[a]	24.31 ± 8.62[c]	16.05 ± 4.83[a]	22.54 ± 7.96[b]	0.12
VSS (mg/l)	13.10 ± 6.14[ab]	14.62 ± 5.84[c]	10.91 ± 4.58[a]	16.53 ± 6.32[bc]	4.05
pH	7.46 ± 0.26[ab]	7.78 ± 0.31[ab]	7.39 ± 0.27[a]	7.62 ± 0.11[b]	3.86

trations has been reported either for pure cultures [8] or for non-adapted and adapted enrichment cultures [6]. Notwithstanding, AOB seem to present a lower sensitivity to high salt concentrations than NOB [9].

The submerged fixed bed biofilm reactor (SFBBR) for wastewater treatment is an alternative to the traditional activated sludge system [10]. Submerged fixed film technology has many advantages, for instance a long sludge retention time, prevention of washout of biomass, and better process stability in terms of withstanding shock loadings or short-term disturbing effects [11]. Other advantages are the simplest control and maintenance, low-energy requirements, low-operating costs and minimized odors and noise [12–14]. Intensive research in the field of biological wastewater treatment showed that biofilms are often more efficient for water purification than conventional suspended activated sludge for the removal of organic matter and nitrogen from wastewater through the biological process of nitrification–denitrification [15].

A limited number of studies are available in the literature addressing nitrifying microbial communities in submerged fixed bed bioreactors (SFBBRs) treating saline wastewater.

In order to understand the AOB communities of biological wastewater treatments, molecular methods based on the sequencing of partial 16S rRNA genes amplified from DNA extracted from environmental samples have been used to reveal intrinsic genetic biodiversity [14]. Furthermore, in recent years new technologies have been developed, such as the second-generation high-throughput sequencing, which can elucidate the characters of microbial community more completely and accurately [16,17]. Pyrosequencing, developed by Roche Life Science, was the first second-generation DNA sequencing platform to be commercially available [18] and it has been used in recent years to elucidate the biodiversity of microbiota involved in important wastewater processes such as nitrogen transformations [19,20]. The aim of this work was to evaluate the effect of saline wastewater on the nitrogen removal process and the community structure of nitrifying bacteria in biofilms developed in a SFBBR, using a pyrosequencing approach targeting the *amoA* gene encoding ammonia monooxygenase.

2. Materials and methods

2.1. Descriptions of the pilot-scale experimental plant and operating conditions

The SFBBR used in this study and the operational conditions were described in full detail in a previous work [12]. Briefly, the SFBBR consisted of a cylindrical methacrylate bioreactor of 0.15 m internal diameter and 0.65 m height, packed with porous plastic carriers, Bioflow 9® (RVT Company, Knoxville, TN, USA), with a surface of 800 m^2/m^3 and a bulk density of 145 kg/m^3 was used as support material for the formation of the biofilm. Air was supplied by a diffuser placed on the bottom of the reactor to achieve a concentration of 6 mg O_2/L. The bioreactor was operated with urban sewage water collected from the primary settling tank of the municipal wastewater treatment plant "EDAR SUR" (EMASAGRA S.A., Granada, Spain).

Four different working salt concentrations were used: i.e., the influent was unamended (named experiment E0) or amended with NaCl (3.7, 24.1 and 44.1 g/L; named experiments E3.7, E24.1 and E44.1, respectively) in order to cover a broad range of salt concentration, as performed by other authors [12,22,23]. The final conductivity of the influents assayed was as follow: 1.5 mS for experiment E0; 12 mS for experiment E3.7; 24 mS for experiment E24.1; and 48 mS for experiment E44.1. These four different experimental conditions were maintained for 45 days each, divided in 3 cycles of 15 days. At the end of each cycle, a backwashing of the biofilter was required due to clogging.

All experiments were carried out with the same inflow rate (50 mL/min), HRT (3.8 h), temperature (20 °C) and air flow rate, according to a previous study [12].

2.2. Physic-chemical analysis

Biological oxygen demand at 5 days (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS) and volatile suspended solids (VSS) were determined according to standard methods for the examination of waste and wastewater [21]. Chloride interference in chemical analysis was avoided by means of silver nitrate according to Ramos et al. [22].

The pH value was monitored using a Crison pH 25 pH-meter (Crison instruments S.A., Barcelona, Spain). Influent and effluent water samples were obtained daily for analytical studies and all the measurements were taken in triplicate.

Table 1 summarizes the characterization of the influents and effluents of the four experiments conducted in the SFBBR (average values of BOD_5, COD, TSS, VSS and pH). As previously reported [12], increasing salinity in the influent significantly reduced the efficiency of organic matter removal (COD and BOD_5) by the SFBBR.

2.3. Monitoring of ammonium, nitrate and nitrite concentrations

For ammonium, nitrate and nitrite analyses, water samples were filtered through 0.22 μm membrane filters (HAWP; Millipore Massachusetts, USA) and subsequently quantified by ion chromatography (IC) [22] using conductivity detection (Dionex® DX-300; Dionex Corporation, Sunnyvale, USA). A Metrosep ASUPP5 column