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Influence of salinity on fungal communities in a submerged fixed bed bioreactor for wastewater treatment

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HIGHLIGHTS

- The influence of salt (NaCl) on fungal communities was studied in a SFBBR.
- Fungal communities' structure was studied by DGGE and 454 pyrosequencing.
- None of the 30 OTUs detected was found at all salinities tested.
- *Basidiomycota* populations were not detected at high salinity (44.1 g NaCl/L).
- The relative abundance of *Ascomycota* increased as wastewater salinity increased.

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ABSTRACT

Salinity is known to influence the performance of biological wastewater treatment plants. While its impact on bacterial communities has been thoroughly studied, its influence on fungal communities has been largely overlooked. To address this knowledge gap, we assessed the effect of saline influents (0, 3.7, 24.1 and 44.1 g NaCl/L) on the community structure and diversity of fungi in a submerged fixed bed bioreactor (SFBBR). For this purpose, denaturing gradient gel electrophoresis (DGGE) and 454-pyrosequencing of PCR-amplified fungal 18S rRNA gene fragments and ITS regions, respectively, were used. Significant differences in the fungal community structure were found in relation to the NaCl concentration. Fungal diversity increased as salinity increased to a concentration up to 24.1 g NaCl/L, but was significantly reduced at 44.1 g NaCl/L. *Basidiomycota* dominated the fungal community in the absence of NaCl but decreased in relative abundance with increasing salinity, being replaced progressively by *Ascomycota*.

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1. Introduction

Salinity is regarded as an often encountered stress factor in wastewater treatment plants (WWTPs) [1,2]. In the last decades, the influx of saline wastewater to WWTPs has increased [3,4]. Some industries such as dairy, olive oil, seafood processing, meat and fish canning, aquafarming, tannery, petroleum, fertilizers, and production of pharmaceuticals and other chemicals, discharge wastewater bearing high salt (NaCl) concentrations [5–7]. The quality of these saline industrial wastewaters may vary seasonally depending on their production rates. For instance, fish canning wastewater bears concentrations of Na^+ ions ranging from 0.086 to 2.120 g/L [8], and streams from tannery industries can contain as much as 80 g/L NaCl [5]. Urban wastewater may also be affected by these saline industrial wastewaters or other sources. For instance, the use in some countries of saline water for toilet flushing due to the scarcity of fresh water raises the salinity that reaches WWTPs [1].

The effects of salinity on the performance of biological WWTPs has been evaluated in several studies [1,5–7,9,10], which concluded that salinity of wastewater over 1% (wt/v) may display adverse effects on treatment performance (organic matter, N and P removal), also influencing significantly the bacterial diversity and community structure. Highly saline organic wastewaters are often poorly biodegraded in conventional WWTPs due to the toxic effect of their sodium content on biomass that has not been previously adapted to saline conditions [7]. The high concentrations of

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salt can cause cell plasmolysis due to the dramatic increase in osmotic pressure and changes on microbial metabolism [5].

An alternative to the conventional activated sludge WWTPs are submerged fixed bed bioreactors (SFBBRs), based on the development of microbial biofilms on a support material [11]. SFBBRs are often more efficient for water purification than conventional suspended activated sludge systems due to their simpler mechanical configuration, smaller footprint, lower costs, longer retention times, endurance of high organic loading rate, low-energy requirements and better process stability in terms of withstanding shock loadings or short-term disturbing effects [12,13]. Biofilm systems also protect microorganisms in hostile environments, characterized e.g. by the presence of antimicrobial agents, UV light and other stressors [14]. The complexity of the multispecies microbial communities in biofilms is largely dependent on environmental factors. Salinity has been described as a major factor regulating bacterial and fungal composition and diversity across many different habitats [2,15].

Among the different aerobic biological WWTPs, fungi were found to play an important role in trickling filters, where they contributed to COD removal, nitrification and denitrification [16]. Da Silva et al. [17] advocated the use of fungi in WWTPs because these microorganisms appeared to show higher degradation rates of organic matter than bacteria, with a better ability to degrade complex polymers such as cellulose, hemicellulose and lignin. These observations endorse the need to investigate more closely the fungal diversity in WWTPs and to integrate these organisms systematically in the treatment processes to exploit their possible bioconversion potential. Knowledge on the mechanisms by which operational variations may influence the community structure of fungi is also crucial for the optimization of nutrient removal rates in WWTPs and the implementation of process control strategies [18,19].

In this study, we examined the effect of increasing salinity on fungal communities in a SFBBR system treating urban wastewater amended with four different NaCl concentrations. Due to the complexity of fungal biodiversity [20,21], a combination of two molecular methods was applied. The diversity of the fungal communities was evaluated firstly using denaturing gradient gel electrophoresis (DGGE) fingerprinting of PCR-amplified 18S rRNA gene fragments, and secondly by 454-pyrosequencing of the internal transcribed spacer (ITS) region. 18S rRNA and ITS sequences were chosen as phylogenetic markers since they were previously used in other studies to analyze the composition of fungal communities in WWTPs [19]. The 18S rRNA gene sequences are recommended targets in molecular methods for the study of diversity of fungi [20], but display a poor species-level resolution [22], while the ITS region has the highest probability of successful identification for the broadest range of fungi and was proposed as a universal DNA barcode marker for fungi [22].

2. Materials and methods

2.1. Description of the experimental reactor, operating conditions and biofilm sampling

The pilot-scale SFBBR (Fig. 1) and the operating conditions (Table 1) used for this study were described in full detail in Cortés-Lorenzo et al. [12]. In short, the SFBBR system consisted of one methacrylate cylindrical column with a bed size of 65 cm height and 15 cm diameter. A porous plastic carrier, Bioflow 9®, was used as support material. Air was supplied by a diffuser placed at the bottom of the reactor to achieve a concentration of 6 mg O_2/L. The reactor was operated with domestic wastewater, coming from the primary settling tank of the municipal WWTP "EDAR

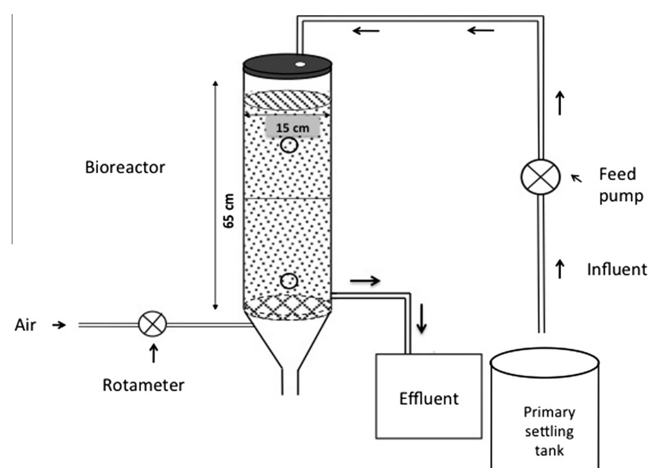

Fig. 1. Diagram of the submerged fixed bed biofilm reactor (SFBBR) used in this study.

Table 1
Operational conditions in the submerged fixed-film bioreactor during the experiments.

Operating conditions	
Days of operation	45
HRT	3.8 h
Temperature	20 °C
Inflow rate	50 mL/min
Air	6 mg O_2/L

SUR" (EMASAGRA S.A., Granada, Spain). The SFBBR was inoculated with real urban wastewater which was recirculated until stable biofilm formation. The experiments were carried out with an inflow rate of 50 mL/min, an HRT of 3.8 h, and a constant temperature of 20 °C.

The experimental work on the SFBBR was divided in four stages (experiments 1, 2, 3 and 4) using increasing concentrations of NaCl. Each stage lasted 45 days and consisted of three working cycles of 15 days. At the end of each cycle, a backwash cleaning was performed to avoid clogging of the biofilter. The influent wastewater was unamended (experiment 1) or amended with NaCl concentrations ranging from 3.7, 24.1 and 44.1 g/L (experiments 2, 3 and 4, respectively). The final conductivity of the influents was 1.5 mS for experiment 1, 12 mS for experiment 2, 24 mS for experiment 3 and 48 mS for experiment 4.

Table 2 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) [12]. The average characteristics were analyzed in accordance with standard methods [23]. As previously reported [12], increasing salinity in the influent significantly reduced the efficiency of organic matter removal (COD and BOD_5) by the SFBBR.

Biofilm samples were collected from the SFBBR as described previously [12] on days 2, 5, 9 and 12 of the third working cycle of every experiment, in order to assess the evolution of the fungal biodiversity. The samples from experiment 1 were named Fung1.1 (day 2), Fung1.2 (day 5), Fung1.3 (day 9) and Fung1.4 (day 12), samples from experiment 2 were named Fung2.1, Fung2.2, Fung2.3 and Fung2.4, and accordingly for the other two experiments.

2.2. DNA extraction, PCR amplification of partial fungal 18S rRNA genes and DGGE analysis

DNA was extracted from biofilm samples using the FastDNA kit and the FastPrep24 apparatus (MP-BIO, Germany). The DNA

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