ELSEVIER

Steady-state inhibition model for the biodegradation of sulfonated amines in a packed bed reactor

Research Paper

Cleotilde Juárez-Ramírez[1], Juvencio Galíndez-Mayer[1], Nora Ruiz-Ordaz[1], Oswaldo Ramos-Monroy[1], Fortunata Santoyo-Tepole[1] and Héctor Poggi-Varaldo[2]

[1] Laboratorio de Bioingeniería de la Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional, Carpio y Plan de Ayala S/N, Casco de Santo Tomás, Miguel Hidalgo, 11340 Ciudad de México, Distrito Federal, Mexico
[2] Laboratorio de Biotecnología Ambiental del Centro Nacional de Estudios Avanzados del Instituto Politécnico Nacional, Av. Instituto Politécnico Nacional 2508, San Pedro Zacatenco, Gustavo A. Madero, 07360 Ciudad de México, Distrito Federal, Mexico

Aromatic amines are important industrial products having in their molecular structure one or more aromatic rings. These are used as precursors for the synthesis of dyes, adhesives, pesticides, rubber, fertilizers and surfactants. The aromatic amines are common constituents of industrial effluents, generated mostly by the degradation of azo dyes. Several of them are a threat to human health because they can by toxic, allergenic, mutagenic or carcinogenic. The most common are benzenesulfonic amines, such as 4-ABS (4-aminobenzene sulfonic acid) and naphthalene sulfonic amines, such as 4-ANS (4-amino naphthalene sulfonic acid). Sometimes, the mixtures of toxic compounds are more toxic or inhibitory than the individual compounds, even for microorganisms capable of degrading them. Therefore, the aim of this study was to evaluate the degradation of the mixture 4-ANS plus 4-ABS by a bacterial community immobilized in fragments of volcanic stone, using a packed bed continuous reactor. In this reactor, the amines loading rates were varied from 5.5 up to 69 mg L^{-1} h^{-1}. The removal of the amines was determined by high-performance liquid chromatography and chemical oxygen demand. With this information, we have studied the substrate inhibition of the removal rate of the aromatic amines during the degradation of the mixture of sulfonated aromatic amines by the immobilized microorganisms. Experimental results were fitted to parabolic, hyperbolic and linear inhibition models. The model that best characterizes the inhibition of the specific degradation rate in the biofilm reactor was a parabolic model with values of $R_{XM} = 58.15 \pm 7.95$ mg $(10^9$ cells h$)^{-1}$, $K_s = 0.73 \pm 0.31$ mg L^{-1}, $S_m = 89.14 \pm 5.43$ mg L^{-1} and the exponent $m = 5$. From the microbial community obtained, six cultivable bacterial strains were isolated and identified by sequencing their 16S rDNA genes. The strains belong to the genera *Variovorax*, *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Nocardioides* and *Microbacterium*. This microbial consortium could use the mixture of aromatic amines as sources of carbon, nitrogen, energy and sulfur.

Introduction

Aromatic amines are important industrial products that have in their molecular structure one or more aromatic rings. These are used as precursors for the synthesis of dyes, adhesives, pesticides, rubber, fertilizers and surfactants [1]. Sulfonated aromatic amines are common constituents of industrial effluents [2], mostly generated by the degradation of the sulfonated azoic dyes [3,4], hazardous to human health, because they can be toxic, allergenic, mutagenic or carcinogenic [5,6]. The most common amines are

Corresponding authors. Juárez-Ramírez, C. (cleotildejr@prodigy.net.mx), Galíndez-Mayer, J. (juvenciogm@yahoo.com, cmayer@encb.ipn.mx)

http://dx.doi.org/10.1016/j.nbt.2014.07.010

the benzene sulfonic acids, such as 4-ABS (4-aminobenzene sulfonic acid) and the naphthalene sulfonic acid, such as 4-ANS (4-amino naphthalene sulfonic acid). The presence of at least one sulfonic group, makes these compounds highly resistant to biological attack [7–9], but also, their solubility is increased; thus, they can easily reach aquatic environments through treated or untreated industrial discharges. Sulfonated aromatic amines have been found in rivers of Italy, Germany and Spain [10–13], also in Mediterranean coastal waters [14]. This means that these compounds are not efficiently removed in the wastewater treatment plants.

Some researchers have evaluated the degradation of 4-ABS using pure cultures, such as *Panonibacter* sp. [1], a co-culture of *Ralstonia* sp. PBA and *Hidrogenophaga* sp. PBC [8]; using microbial consortia [5], or acclimatized activated sludge [3]; however, a toxic effect of 4-ABS is observed in all the systems, even for the microorganisms that use it as carbon and energy source.

About 4-ANS, few works concerning its biodegradation are reported, and only with processes using microbial consortia, a partial degradation of this aromatic amine have been achieved [15]. Recently, Juárez *et al*. [16], reported the effect of the culture medium composition on the degradation of 4-ANS by a bacterial community immobilized on fragments of volcanic stone. In 2013, Koupaie *et al*. [17], reported 80% of degradation of the 4-ANS generated during Acid Red 18 biodegradation, using activated sludge. Commonly, mixtures of 4-ABS and 4-ANS, as degradation byproducts of sulfonated azo dyes, are present in industrial effluents. The mixtures of toxic compounds are frequently more toxic or inhibitory than the individual compounds; therefore, the aim of this research was to evaluate the degradation of 4-ANS and 4-ABS, using a bacterial community immobilized in a packed-bed reactor, and establish a substrate inhibition model describing the effect of the concentration of the sulfonated aromatic amines on their specific removal rate.

Material and methods

Reagents. 4-ABS and 4-ANS were analytical reagents from Sigma–Aldrich, USA, with 99% and 97% purity, respectively. The HPLC solvents were purchased from J.T. Baker, USA.

Culture medium. The composition of the mineral salts (MS) medium in mg L^{-1} was: K_2HPO_4, 200; $MgCl_2$, 80; $CaCl_2$, 20. The sulfonated aromatic amines 4-ABS and 4-ANS (50 mg L^{-1} each one), were added as a source of carbon, nitrogen, sulfur and energy.

Enrichment of a microbial community able to grow in a mixture of 4-ANS and 4-ABS

Samples of soil from a site near a textile industry and samples of sediments from the banks of the dam of Requena, located in the State of Hidalgo, Mexico, were collected and immediately processed in the laboratory by inoculating one gram of sample in 250 mL flasks with 50 mL of MS medium, containing a mixture of 4-ANS and 4-ABS, 25 mg L^{-1} each one. The flasks were incubated at room temperature with constant stirring (60 rpm) during 72 h. The removal of aromatic amines was spectrophotometrically quantified at 238 nm for 4-ANS and 248 nm for the 4-ABS. Aliquots of 10 mL were successively transferred to Erlenmeyer flasks containing new MS medium plus the mixture of aromatic amines, repeating the same incubation procedure. After eighteen transfers, the bacterial community showing the highest removal rate of amines was selected.

Isolation and identification of the predominant bacteria in the microbial community

Of the previously selected community, decimal dilutions of a sample of suspended cells were prepared. Aliquots of 200 μL were distributed in nutrient agar plates. The plates were incubated at 30°C during 48 h. The types of cultivable bacteria present in the microbial community were estimated by observing differences in colonial morphology. The isolated bacteria were cryopreserved in glycerol at −70°C.

Each isolate was propagated in Luria-Bertani medium; the cell packages obtained after centrifugation were used for DNA extraction. By PCR amplification of the extracted and purified DNA, amplicons close to 1400 bp of the 16S rDNA were obtained, using 8FPL (5′AGT TTG ATC CTG GCT CAG 3′) and 13B (5′AGG CCC GGG AAC GTA TTC AC 3′) oligonucleotides [18]. The amplicons were purified, sequenced (Macrogen Co., Korea) and compared with the known sequences of bacterial 16S rDNA at NCBI Genbank.

Packed-bed reactor (PBR)

The packed bed reactor was a bubble column packed with fragments of a porous volcanic stone named tezontle in Mexico. The characteristics of the porous material were obtained according to Gomez-De Jesús *et al*. [19]. The particle volume was calculated and used to estimate the equivalent diameter d_p of the volcanic stone particles considering porous fragments as ellipsoidal bodies, with three characteristic radii. The average d_p value was 7.4 ± 2.7 mm.

The column was made of glass and has two entries in the bottom, one for the fresh culture medium and another for the air. A sintered glass plate diffuser (with pore diameter 40–100 μm) is located at the bottom of the PBR. On the upper side, the column has a lateral port for the air and outflowing medium that keeps the operating liquid volume constant. The cover of the column reactor has ports for inoculation and venting (Fig. 1). All the experiments

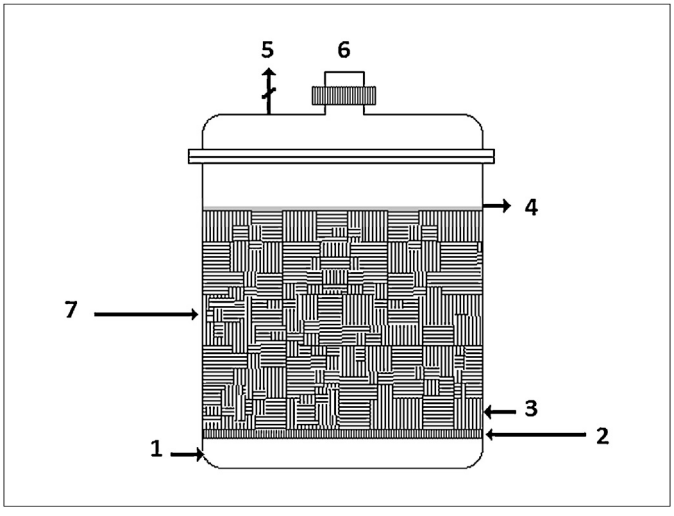

FIGURE 1

Packed-bed column reactor. (1) Air input; (2) sintered glass plate; (3) liquid input; (4) liquid and gas output; (5) air venting; (6) inoculation port; (7) porous support for microbial biofilm.