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Effect of an azo dye on the performance of an aerobic granular sludge sequencing batch reactor treating a simulated textile wastewater

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ABSTRACT

This study analyzed the effect of an azo dye (Acid Red 14) on the performance of an aerobic granular sludge (AGS) sequencing batch reactor (SBR) system operated with 6-h anaerobic-aerobic cycles for the treatment of a synthetic textile wastewater. In this sense, two SBRs inoculated with AGS from a domestic wastewater treatment plant were run in parallel, being one supplied with the dye and the other used as a dye-free control. The AGS successfully adapted to the new hydrodynamic conditions forming smaller, denser granules in both reactors, with optimal sludge volume index values of 19 and 17 mL g^{-1} after 5-min and 30-min settling, respectively. As a result, high biomass concentration levels and sludge age values were registered, up to 13 gTSS L^{-1} and 40 days, respectively, when deliberate biomass wastage was limited to the sampling needs. Stable dye removal yields above 90% were attained during the anaerobic reaction phase, confirmed by the formation of one of the aromatic amines arising from azo bond reduction. The control of the sludge retention time (SRT) to 15 days triggered a 30% reduction in the biodecolorization yield. However, the increase of the SRT values back to levels above 25 days reverted this effect and also promoted the complete bioconversion of the identified aromatic amine during the aerobic reaction phase. The dye and its breakdown products did not negatively affect the treatment performance, as organic load removal yields higher than 80% were attained in both reactors, up to 77% occurring in the anaerobic phase. These high anaerobic organic removal levels were correlated to an increase of *Defluviicoccus*-related glycogen accumulating organisms in the biomass. Also, the capacity of the system to deal with shocks of high dye concentration and organic load was successfully demonstrated. Granule breakup after long-term operation only occurred in the dye-free control SBR, suggesting that the azo dye plays an important role in improving granule stability. Fluorescence *in situ* hybridization (FISH) analysis confirmed the compact structure of the dye-fed granules, microbial activity being apparently maintained in the granule core, as opposed to the dye-free control. These findings support the potential application of the AGS technology for textile wastewater treatment.

1. Introduction

With the increasing demand for textile products, the textile industry wastewater represents one of the main sources of water pollution problems worldwide, mainly due to high organic loads and recalcitrant dyes (Dos Santos et al., 2007). Textile dyes are responsible for the presence of color in textile wastewater, impairing light penetration and compromising ecosystems in the receiving water media. Bacterial decolorization of azo dyes, the

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main type of synthetic textile dyes used, is generally accomplished through anaerobic azo bond cleavage with colorless aromatic amine formation. However, these potentially toxic breakdown intermediates are generally not further degraded anaerobically.

In this context, anaerobic-aerobic sequencing batch reactors (SBRs) with flocculent activated sludge have been proposed for complete biodegradation of azo dyes through azo bond reduction in the anaerobic phase, with concomitant color removal, followed by aromatic amine mineralization in the subsequent aerobic step (Van der Zee and Villaverde, 2005). Nevertheless, despite the notable success in the anaerobic decolorization stage (Lourenço et al. 2000; Albuquerque et al. 2005), information regarding the fate of the breakdown aromatic amines during the aerobic stage, when available, revealed that most of these amines were not degraded (Van der Zee and Villaverde, 2005). This difficulty in mineralizing azo dye reduction products under aerobic conditions has generally been attributed to the lack of an adequate microbial population capable of metabolizing such compounds (Lourenço et al. 2009). Given this troubling scenario, there is an urgent need for effective, environmentally friendly and economically attractive technologies for textile wastewater treatment.

Furthermore, intrinsic operational problems of flocculent activated sludge systems, such as poor settling properties, compromise the treatment efficiency and lead to large footprint requirements. The use of aerobic granular sludge (AGS) has been recently suggested to overcome these problems. The AGS technology, with near spherical structures of self-aggregated microorganisms formed under specific SBR operational conditions (Beun et al., 2002), has been implemented in several domestic and industrial treatment plants, being often referred as the next generation of wastewater treatment (Giesen et al., 2013). In addition to the outstanding settling characteristics of AGS, the co-existence of aerobic and anoxic-anaerobic zones within the granules (Winkler et al., 2013) and their resistance to high organic loads and toxic compounds (Giesen et al., 2013) reinforce the promising application of the AGS technology for textile wastewater treatment, though practical demonstration has scarcely been reported (Muda et al., 2010). Moreover, the operational sludge retention time (SRT) flexibility of AGS systems enables the presence of a more diverse microbial community within the SBR, namely slow-growing populations, whose activity may be advantageous for the degradation of recalcitrant compounds (Clara et al., 2005).

In this context, the aim of this work was to study the effect of an azo dye in the characteristics and performance of AGS in an anaerobic-aerobic SBR system treating synthetic textile wastewater. The performance of two AGS SBRs run in parallel (one supplied with the dye and the other used as a dye-free control) was evaluated in terms of AGS stability, microbial community, azo dye and intermediate aromatic amine biodegradation and chemical oxygen demand (COD) removal efficiency. The capacity of the system to deal with sudden, high dye concentrations and organic loads in the feed was also evaluated.

2. Materials and methods

2.1. Chemicals

2.1.1. Carbon source and dye stock solutions

A starch-based sizing agent used in the textile industry, Emsize E1 (Emsland-Stärke GmbH, Germany), was used as carbon source. The stock solution (100 g L^{-1}) was prepared by hydrolyzing a solution of Emsize E1 in distilled water, in alkaline conditions, based on a set of desizing conditions indicated by the manufacturer, as described by Lourenço et al. (2000).

The azo dye stock solution was prepared by dissolving Acid Red 14 (AR14, Chromotrope FB, Sigma–Aldrich, 50% dye content) in distilled water to a final concentration of 3.0 g L^{-1}.

2.1.2. Synthetic wastewater composition

The synthetic textile wastewater used as feed solution was prepared by diluting the carbon source stock solution in distilled water to a COD content of 1000 mg O_2 L^{-1} (1.15 g L^{-1} Emsize E1), and supplementing it with pH buffering phosphates and nutrients to the following concentrations: 2310 mg L^{-1} $Na_2HPO_4\cdot12H_2O$, 762 mg L^{-1} KH_2PO_4, 143 mg L^{-1} NH_4Cl, 22.5 mg L^{-1} $MgSO_4\cdot7H_2O$, 27.5 mg L^{-1} $CaCl_2$, 250 μg L^{-1} $FeCl_3\cdot6H_2O$, 40 μg L^{-1} $MnSO_4\cdot4H_2O$, 57 μg L^{-1} H_3BO_3, 43 μg L^{-1} $ZnSO_4\cdot7H_2O$, 35 μg L^{-1} $(NH_4)_6Mo_7O_{24}\cdot4H_2O$. All salts were analytical grade. In this feed solution the COD:N:P mass ratio was 100:3.7:37. The low N supply was used to avoid the occurrence of nitrification. For the carbon shock load assays the Emsize E1 and NH_4Cl concentrations in the feed were the triple of those indicated above.

2.2. SBR setup and operation

Two bubble-column SBRs with a working volume of 1.5 L (height/diameter ratio of 2.5) were seeded with AGS harvested from the Nereda® demonstration plant at the Frielas domestic wastewater treatment plant (WWTP), Portugal, which is operated in a sequencing batch mode with an anaerobic filling stage followed by an aerobic reaction phase.

Mechanical mixing (70 rpm) was provided by magnetic stirrers and aeration (2 v.v.m.) was supplied by air compressors via a porous membrane diffuser at the bottom of each bioreactor. The synthetic wastewater was fed into both SBRs at the bottom with an exchange ratio of 50% (effluent withdrawal at mid-height), the volumetric organic loading rate (OLR) being 2.0 kg COD m^{-3} d^{-1}. While one bioreactor was used as a dye-free control (SBR1), the other was fed with the azo dye stock solution (SBR2) to an initial dye concentration of 20 mg L^{-1} at the onset of the reaction phase.

The SBRs were run in parallel for 102 days, comprising five experimental periods (I–V), characterized in Table 1. Deliberate biomass wastage was limited to sampling, except during period II, when the SRT was controlled at 15 days through daily biomass purging from SBR mixed liquor. To test the capacity of the system to deal with high dye and organic loads, the initial dye concentration in SBR2 was increased to 60 mg L^{-1} from day 91 on (period IV), and the volumetric OLR was increased to 6.0 kg COD m^{-3} d^{-1} from day 98 on (period V).

Reactors were operated at room temperature (23 °C, on average) in a sequencing batch mode with 6-h cycles, of which the first 1.5 h of reaction were anaerobic (with mechanical mixing), followed by 3.5 h of aeration. The static filling period was fixed at 18 min, the dye stock solution being added at the top of SBR2 during the last 1.5 min of this period. The settling and effluent withdrawal times were 5 min and 1 min, respectively. The mixed liquor pH value (uncontrolled) varied within the 6.5–6.7 range along each cycle.

2.3. Analytical methods

2.3.1. Physical-chemical parameters

Total suspended solids (TSS), volatile suspended solids (VSS), COD and pH were determined according to standard procedures (APHA, 1995). Sludge volume index (SVI) was determined by measuring the volume occupied by the sludge settled from 1 L of mixed liquor after settling times of 5 and 30 min in an Imhoff cone (SVI_5 and SVI_{30}, respectively), and dividing it by the mixed liquor TSS value. Biomass morphological analysis was carried out using a transmission light microscope (BA200, Motic) fitted with a digital camera and respective software (Moticam 2, Motic). The proportion