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Anaerobic treatment of azo dye *Acid Orange 7* under batch conditions

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Abstract

Degradation of the azo dye *Acid Orange 7* (AO7) in batch anaerobic unstirred assays is described. Experiments were carried out: (i) under abiotic conditions, (ii) with active biomass using only the dye as organic substrate, and (iii) with the dye and different cosubstrates. Non-adapted biomass was used. The results obtained indicate that AO7 was only removed in the presence of active biomass, the removal rates being higher in the presence of a cosubstrate. The highest removal rates were obtained with a high concentration of glucose, $2\,g\,l^{-1}$, which indicates that apart from the positive influence of the presence of an excess of reducing equivalents, the improvement of mass transfer conditions in the medium as a consequence of the high biogas production is also a key topic. AO7 yields sulphanilic acid (SA), which was not further degraded and was accumulated in the medium in stoichometric amounts. The other compound resulting from AO7 breakdown, 1-amino-2-naphthol (1A2N), was not detected most likely because of its low stability. However, the detection of 1,2-naphthoquinone (12NQ), a compound generated after the oxidation of 1A2N gives evidence of this mechanism.

Keywords: Azo dyes; Batch assays; Anaerobic treatment; Aromatic amines; Reducing equivalents; Mineralisation

1. Introduction

The generation of highly coloured wastewaters by the textile industry nowadays constitutes an important environmental problem. Colour usually appears as the result of the presence of low concentrations of specific compounds, such as azo dyes, which represent the commonest group of dyes used. Above 60–70% of more than 10 000 dyes used in the textile industry are azo dyes [1]. These compounds have an azo bond (R_1–N=N–R_2), where R_1 and R_2 are aromatic groups which, in some cases, can be substituted by sulphonated groups. Although azo dyes can not be easily degraded under aerobic conditions, it has been shown that the azo bond can be reduced anaerobically [1–4], which causes colour removal. However, the products obtained from the cleavage of the azo bond are aromatic amines, which are considered carcinogenic compounds [5]. Aromatic amines are quite stable in anaerobic environments. Further mineralisation of these compounds has been reported under aerobic conditions [6–8], although autoxidation can also occur [9].

The non-specific nature of azo dye reduction has been established by many researchers [2,10,11]. Gingell and Walker [12] proposed that biological azo dye reduction is a non-specific chemical reaction between azo dyes and reduced flavins that are generated by cytosolic flavin-dependent reductases (flavin reductases). The ability of these enzymes to act in vitro as azoreductases has been reported [13]. In fact, the role of reduced flavins (FMN and FADH) has been shown as accelerators of the azo dye reduction by acting as electron carriers [2,6]. Also, other enzymatic cofactors, such as NADH or NADP(H) have been reported to be active electron donors for the reduction of tartrazine [14]. Artificial redox mediators, such as benzyl viologen [2,15–17] or different quinones [16,18] have been reported to accelerate azo dye

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reduction. The inhibition of the azo dye reduction in the presence of oxygen [19] or other electron acceptors like nitrite or nitrate [1,20] is explained by this theory because of their higher oxidative character.

It was reported that cell extracts or membrane-permeabilized cells reduce azo dyes more efficiently than entire cells, especially in the case of sulphonated azo dyes [20,21]. The flavin reductases are located in the cytoplasm of the cells [13], which suggests that anaerobic reduction of azo dyes is an intracellular process and the permeation of azo dyes across the cell membrane is a rate-limiting factor [21].

On the contrary, using different bacterial cultures, other authors found similar removal rates for polymeric azo dyes and other analogue compounds with much simpler structures [15], an extracellular mechanism for azo dye reduction being proposed. Besides, a study with different azo dyes showed that azo dye removal rate was a function of the dye redox potential. In this way, Amaranth was reduced more quickly than *Acid Orange 7* in spite of the presence of three sulphonic groups in its structure [17]. Kudlich et al. [16] reported the presence of azoreductase activity in the membrane cells of *Sphingomonas* sp. BN6. They suggested that anthraquinone sulphonate redox mediators reduce the dye extracellularly and are regenerated by the cell surface enzyme.

Dyes exhibit low toxicity to mammals and aquatic organisms [22]. Only 2% of about 300 colorants tested by Clarke and Anliker [23] had an LC_{50} for fish lower than 1.0 mg l^{-1}, whereas around 96% of compounds had values above 10 mg l^{-1}.

The potential problem of azo dyes regarding human toxicity is associated with the type of intermediates used in their synthesis and appears only after the reduction and cleavage of the azo bond to give aromatic amines. Several azo dyes used as colorants for food, drugs and cosmetics can be reduced by cell suspensions of predominant intestinal anaerobes [2], so it can be assumed that the ingestion of certain azo dyes is a risk for human health. In this sense, 1-amino-2-naphthol, produced by the reduction of *Acid Orange 7*, has been reported to induce bladder tumours [5]. In the same way, the azo dye Amaranth has been shown to be carcinogenic for rats [24]; this dye was widely used as a food colorant in many countries [25]. Azo dyes based on benzidine or 2-napthylamine are considered genotoxic and during the past decades, most of the research into the dye manufacturing industry was focussed on the production of an alternative product for these compounds.

Standardized bioassays with luminescent bacteria (commonly applied as the commercially available Microtox®) showed that in five of the six azo dyes tested, anaerobic treatment caused an increase in the toxicity [26]. In contrast, anaerobic treatment of azo dyes with a nitro group can produce detoxification. Thus, *Mordant Orange 1* is much more toxic for methanogenic bacteria than the aromatic amines generated in its reduction [3]. *Mordant Yellow 12* is less toxic than *Mordant Orange 1* although they have the same structure with the difference of an amino group instead of a nitro group.

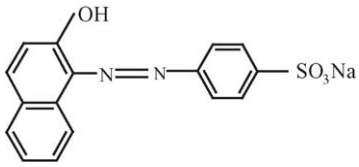

Fig. 1. Chemical structure of *Acid Orange 7*.

Also, *Mordant Yellow 12* is less toxic for methanogenic bacteria than the aromatic amines generated in the reduction of the dye [3].

The objective of this work has been to assess the anaerobic biodegradability of *Acid Orange 7* (AO7) under batch conditions. Fig. 1 shows the chemical structure of this dye.

2. Materials and methods

2.1. Biomass

Non-adapted methanogenic granular sludge from a pilot-scale reactor treating wood-processing wastewaters was used. Sludge was previously washed in order to prevent additional organic matter in batch tests. Final biomass concentration in bottles was around 1.0 g VSS l^{-1}.

2.2. Chemicals

Acid Orange 7 (85–95%) and sulphanilic acid (99.9%) were purchased from Sigma (Madrid, Spain). 1-Amino-2-naphthol hydrochloride (90%) was purchased from Aldrich Chemical Company (Madrid, Spain).

2.3. Experimental assays

Batch assays were carried out in 500 ml serum bottles without stirring. Half useful volume was occupied by the liquid phase.

Two different types of biological assays were carried out. In one case the dye was the sole carbon source. In the other case, different cosubstrates were used. The concentrations of AO7 used ranged between 0.06 and 0.57 mM (25–200 mg l^{-1}) in assays without cosubstrate, and between 0.06 and 0.86 mM (25–300 mg l^{-1}) in assays with cosubstrate. In all experiments, a basal media containing the following compounds was used (mg l^{-1}): NH_4Cl (230), K_2HPO_4 (37), $MgSO_4$ (6), $CaCl_2$ (6), $FeCl_2{\cdot}4H_2O$ (2), HBO_3 (0.35), $ZnCl_2$ (0.05), $CuCl_2{\cdot}2H_2O$ (0.038), $MnCl_2{\cdot}4H_2O$ (0.5), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.05), $AlCl_3{\cdot}6H_2O$ (0.09), $CoCl_2{\cdot}6H_2O$ (2), $NiCl_2{\cdot}6H_2O$ (0.092), $Na_2SO_3{\cdot}5H_2O$ (0.164), EDTA (1), Resazurine (0.2) and HCl (1 μl l^{-1}). To ensure pH stability, $NaHCO_3$ was added in a ratio of 1 g g^{-1} COD. Hundred milligrams per litre of $Na_2S{\cdot}9H_2O$ was added to remove dissolved oxygen. The final pH was adjusted to 7.0 ± 0.1. Before start-up, the headspace of the bottles was flushed with a N_2/CO_2 (80:20) mixture