Microbial decolourisation of a reactive azo dye under anaerobic conditions

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Abstract

Water-soluble azo dyes are used extensively in the textile industry and are known to be problematic with respect to the removal of colour from textile waste waters. Under anaerobic conditions azo dyes can be utilised as terminal electron acceptors in microbial respiration, and are reduced and decolourised concurrently with re-oxidation of reduced flavin nucteotides. The roicrobial decolourisation of an azo dye (C.I. Reactive Red 141) was investigated with respect to the kinetic order of azo reduction and rate-controlling factors of the reaction. Decolourisation of C.I. Reactive Red 141 was found to be first order with respect to dye concentration, although increasing the initial dye concentration in the serum bottles resulted in decreasing k values of -0.441/h (100 m*g/l* of C.I. Reactive Red 141), -0.316/h (150 *mg/l)* and -0.252/h (200 *mg/l).* The presence of labile carbon in the anaerobic system was found to be essential in order to obtain an acceptable rate of decolourisation. The k value obtained for decolourisation of the azo dye without a supplemental carbon source (glucose) was -0.012/h, in comparison to a k value of -0.441/h when supplemented withglucose (1 g/l.The presence of nitrate in the anaerobic system was found to inhibit decolourisation, while the presence of sulphate was found to have no discernible effect on the rate of decolourisation. A low redox potential (-450 to -500 mV) was found to be conducive to rapid decolourisation of C.I. Reactive Red 141. A C.I. Reactive Red 141 degradation product was positively identified as 2-arainonaphthalene-l ,5-disulphonic acid, confirming that azo reduction was responsible for decolourisation of the azo dye. A toxicity assay was performed which showed that C.I. Reactive Red 141 was inhibitory to the anaerobic microbial community at concentrations >100 *mg/l,* but that prior exposure of the biomass to the dye increased the resistance to previously inhibitory dye concentrations.

Introduction

Azo dyes account for 60 to 70% of all textile dyestuffs produced and are the most common chromophore of reactive textile dyes. Colouration of textile effluents (in particular red hues) can usually be linked to the presence of water-soluble (reactive) azo dyes in the waste water. It is generally accepted that the aerobic biological processes at conventional treatment works do not substantially decrease the colouration of these effluents, usually resulting in colour contamination of the receiving water body. It is also well known that substantial decolourisation of azo dyes occurs by reduction of the azo bonds and subsequent destruction of the dye chromophore/s, a process that was linked to the activity of anaerobic micro-organisms as early as 1911 (Meyer, 1981). Thus, although aerobic processes are traditionally used for the treatment of high volume liquid effluents, the potential of anaerobic applications should be explored in the field of textile effluent treatment.

A review of the literature shows that research into anaerobic microbial azo reduction was initiated by concern over metabolic products resulting from the reduction of azo food dyes in the mammalian intestine. For this reason, much of the early research focused on the mechanism of azo reduction by intestinal micro-organisms, whereas later studies tended towards environmental applications. One of the first papers to contain a comprehensive investigation into the mechanism of microbial azo reduction was published by Gingell and Walker (1971), using *Streptococcus faecalis* and the azo dye, Red 2G. The researchers proposed that reduced soluble flavins act as electron shuttles to ferry electrons from the flavoproteins of the microbial electron transport chain to the acceptor azo compound. That is, the azo dye acts as an oxidising agent for the reduced flavin nucleotides of the electron transport chain and is reduced and decolourised concurrently with re-oxidation of the reduced flavin nucleotides. Dubin and Wright (1975), investigating the reduction of various azo food colourants by *Proteus vulgaris,* expanded this theory to include a rate-controlling step which involved a redox equilibrium between the dye and an extracellular reducing agent, with the site of reduction being extracellular. That is, the specific reduction potential of the dye was proposed to be the principal rate-limiting factor in azo reduction. Subsequent research by Yatome et al. (1991) showed that the rate of azo reduction was not limited by the specific dye reduction potentials but by the degree of sulphonation of the dyes. These researchers concluded that azo reduction must occur intracellularly, with the rate of permeation of the dye through the cell membrane being the principal rate-limiting factor. Additional evidence of cell permeability as a primary rate-limiting factor, in microbial azo reduction was reported by Mechsner and Wuhrmann (1982), who managed to substantially increase the reduction rates of azo compounds by permeabilising bacterial cells prior to azo reduction. Anaerobic decolourisation of azo dyes has also been reported using micro-organisms such *as Bacillus subtilis* (Horitsu et al., 1977), *Bacillus cereus* (Wuhr-mann et al., 1980), *Pseudomonas cepacia* (Ogawa et al., 1986; and Ogawa and Yatome, 1990), *Pseudomonas stutzeri* (Yatome et al., 1990) and *Aewmonas hydrophilia* (Idaka and Ogawa, 1978; Yatome et al., 1987).

Although the mechanism of microbial azo reduction has been investigated and reported in the literature, many questions arise about the application of this process for the decolourisation of textile effluent. Some salient concerns are the fate and effect of the

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