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Salinity effects on biodegradation of Reactive Black 5 for one stage and two stages sequential anaerobic aerobic biological processes employing different anaerobic sludge

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ABSTRACT

In this study the effect of NaCl, normally found in dye bath wastewaters employing reactive azo dyes, on the performance of sequential anaerobic–aerobic processes for treatment of Reactive Black 5 (RB5) containing media, with concentration in the range 100–500 mg L^{-1}, was investigated. Three possible scenarios of the sequential anaerobic–aerobic process, namely two stage process and one stage processes employing either anaerobic or activated sludge, were considered. The results showed a statistically significant enhancement of the anaerobic decolourisation efficiency as a result of the addition of 30 g L^{-1} NaCl to the RB5 containing media for two stage processes and one stage processes employing anaerobic sludge. NaCl at 30 g L^{-1} concentration also inhibited aerobic colour formation during two stage processes whereas it prevented aerobic decolourisation during one stage processes. HPLC and UV Vis analysis indicated that during anaerobic phase/stage the majority of azo bonds in RB5 molecules cleave whereas the hydrophobicity/MW of the resulting dye reduction metabolites decreases. The same analysis revealed partial mineralisation of RB5 reduction metabolites under aerobic conditions. The results of the present work also showed that the effect of salt on anaerobic decolourisation efficiency, TVFA and methane production was dependent on the exposure history of anaerobic sludge.

1. Introduction

The sequential anaerobic–aerobic biological process has been considered as a promising technology for both decolourisation and COD reduction of azo dye containing wastewaters (van der Zee and Villaverde, 2005). In this process, the azo dye is reduced in the anaerobic stage or phase of the process whereas the resultant dye reduction metabolites are degraded either partially (Ahmad et al., 2010; Lin et al., 2010; Spagni et al., 2010; Bonakdarpour et al., 2011; Hosseini Koupaie et al., 2012; Murali et al., 2013) or totally (Forss and Welander, 2011) in the subsequent aerobic phase of stage. One of the less studied aspect of this process is the effect of salt on the system performance. The study of this aspect, however, is important because significant quantities of salt, mainly in the form of NaCl and to a much lesser extent sodium sulphate, are added to the dye bath when dyeing textiles, particularly cotton ones (Amaral et al., 2014). Depending on the dyeing procedure employed the concentration of these salts in the dyebath effluent can range from 20 g L^{-1} to 80 g L^{-1} (Correia et al., 1994; Lewis, 2001).

There has been limited previous work on the effect of salts on the anaerobic decolourisation performance of processes employing either anaerobic or activated sludge. Regarding the effect of sodium sulphate, Carliell et al. (1994) found no effect for this salt, at concentrations up to 10 mM, on the rate of decolourisation of Reactive Red 141 by anaerobic sludge. On the other hand, Amaral et al. (2014) found that, due to the competition of sulphate with azo dye for electrons, high sulphate concentrations (>300 mg L^{-1} SO_4^{2-}) reduce the rate of anaerobic decolourisation of a real direct azo dye containing textile wastewater.

The nature of the potential effect of sodium chloride on the sequential anaerobic–aerobic process is very different to that of sodium sulphate. Sodium chloride, as a result of increasing the osmotic pressure, usually has a negative effect on the growth and activity of the normal bacterial population found in anaerobic and

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activated sludge, although the severity of the effect can vary from one bacterial group to another (Kokabian et al., 2013). An inhibitory effect of the presence of NaCl on both the anaerobic and aerobic COD removal efficiency is therefore to be expected. However, previous work has shown that the effect of NaCl on anaerobic decolourisation is not as straightforward. For example, Isik and Sponza (2004) working on anaerobic treatment of a simulated wastewater containing RB5, found that NaCl concentrations up to 128 g L^{-1} did not affect the extent of anaerobic decolourisation with partially granulated anaerobic sludge. On the other hand, Willetts et al. (2000) found that NaCl, up to 20 g L^{-1}, did not adversely affect decolourisation by thermophilic sludge, but it temporarily reduced it in the case of mesophilic sludge. A pronounced negative effect of NaCl, for concentrations >50 g L^{-1}, on anaerobic decolourisation has also been reported in studies with activated sludge (Dafale et al., 2008).

Recently, Kokabian et al. (2013) reported that NaCl has a complex time-dependent effect on the rate of anaerobic decolouristaion of RB5 by anaerobic sludge. During the early part of the process NaCl severely inhibited anaerobic dye decolourisation, but during the later stages the inhibitory effect was much less, and at some salt concentrations the effect was actually positive. These authors have also, for the first time, reported an inhibitory or retarding effect of salt on the autooxidation of aromatic amines resulting from anaerobic reduction of Reactive Black 5; however, the effect of this phenomenon on the aerobic colour change during various scenarios of the anaerobic–aerobic process was not considered in this study.

The above survey of literature highlights the need for more studies to further elucidate the complex effect of NaCl on the performance of the sequential anaerobic–aerobic process. For this reason, in the present work the effect of the presence of sodium chloride on the performance of three scenarios of sequential anaerobic–aerobic biological process (namely one stage process employing either anaerobic or activated sludge and two stage process) for the treatment of a synthetic wastewater containing various concentrations of Reactive Black 5 was investigated. Furthermore, the effect of salt on anaerobic methane and VFA production, as well as total DOC removal, was also considered. Finally, the effect of increasing sodium chloride concentration, in the range 10–50 g L^{-1}, on the anaerobic decolourisation of RB5 achieved by various anaerobic sludge – that were pre-exposed to different environments - was also studied.

2. Materials and methods

Reactive Black-5 (purity 55%) was obtained from Sigma–Aldrich (Gillingham, UK). The dye was hydrolysed using 0.1 M NaOH at 100 °C for 10 min followed by neutralization with phosphoric acid (85%) (Libra et al., 2004); this was done to simulate the hydrolysed form of the dye which is usually the dominant form present in dyebath wastewaters. Glucose was used as a co-metabolite for azo dye decolourisation. The inorganic nutrient media used in both the anaerobic and aerobic stage of the experiments is similar to that previously described by Bonakdarpour et al. (2011) except that additionally NaCl at a concentration in the range 0–50 g L^{-1} was also incorporated in the dye media.

Anaerobic sludge was obtained from a conventional sewage sludge digester (Mogden, UK) and maintained in four different two litre batch reactors. All the reactors contained glucose and the inorganic media as described above. One reactor was operated under non-saline conditions whereas the other three were operated for two months at NaCl concentration of 10, 30 and 50 g L^{-1} respectively. Aerobic sludge was obtained from the activated sludge unit of the wastewater treatment plant at Stevensons Dyers in Derbyshire, and was maintained in a 2 L batch reactor. Both the anaerobic and aerobic reactors were kept in a 30 °C room and operated in a sequencing batch mode.

The anaerobic stage or phase of the experiments was conducted using the serum bottle technique developed by Owen et al. (1979) whereas the aerobic stage or phase was carried out in Erlenmeyer flasks. The procedure employed has been described in Bonakdarpour et al. (2011).

In the present study three scenarios of sequential anaerobic–aerobic biological process was considered. The first scenario simulated two stage process runs: in these experiments anaerobic sludge was anaerobically incubated with RB5 containing media with concentrations in the range 100–500 mg L^{-1} and the effluent at the end of anaerobic incubation was then incubated aerobically. In the second and third scenarios, one stage process runs were simulated in which anaerobic (2nd scenario) and aerobic sludge (3rd scenario) were fed with the same RB5 containing media and exposed to alternating anaerobic aerobic environments.

The runs simulating the one- and two stage sequential anaerobic–aerobic processes were initially performed in the absence of salinity and with RB5 concentration (in the range 100–500 mg L^{-1}) as three repeated batch runs until the decolourisation efficiency approached a more or less constant value. The respective sludge at the end of these runs was subsequently used to perform a further batch, but this time with incorporation of 30 g L^{-1} NaCl in the media.

For analysis, 5 ml duplicate samples were taken from each bottle or flask and filtered through 0.4 micron filters. In the case where samples from serum bottles were to be analysed spectrophotometrically or by HPLC, 5 ml of a phosphate buffer (10.86 g L^{-1} $NaH_2PO_4 \cdot 2H_2O$; 5.38 g L^{-1} $Na_2HPO_4 \cdot H_2O$) containing ascorbic acid (200 g L^{-1}) was added immediately to prevent autooxidation of the dye metabolites (van der Zee et al., 2001; Bonakdarpour et al., 2011).

Determination of VSS was performed according to Standard Methods (APHA, 1999). The standard deviation for 5 identical samples was within 1%. The methane content of the biogas was determined using a Shimadzu GC-TCD fitted with a Porapak N column (1500 × 6.35 mm) (Vyrides and Stuckey, 2009). The carrier gas was helium at a flow rate of 50 ml/min. The column, detector and injector temperature were 28, 38 and 128 °C, respectively. The peak areas were calculated and printed out on a Shimazdu Chromatopac C-R6A integrator. Samples of 1 ml were collected using 1 ml plastic syringes (Terumo). The standard deviation for 5 identical samples was within 1%.

Volatile fatty acids (VFAs) were measured with a Shimadzu GC-FID using H-e as a carrier gas, the detector at 250 °C and an SGE capillary column (12 m × 53 mm ID-BP21 0.5 μm) at 80 °C (Vyrides and Stuckey, 2009). The standard deviation for 10 identical samples was 2%. The total VFA (TVFA) values reported are the average from the two serum bottle runs under each condition. Dissolved Organic Carbon (DOC) was measured with a Shimadzu 5050 (Shimadzu, UK) TOC analyser (Bonakdarpour et al., 2011). The standard deviation for 3 samples was 0.5%. Before DOC analysis, the biomass was removed by centrifugation and filtration (0.2 μm). The initial DOC for the runs with and without dye were determined as 676 g L^{-1} and 858 g L^{-1} respectively.

In order to determine the concentration of RB5 and monitor dye degradation metabolites throughout the three scenarios of sequential anaerobic–aerobic process, spectrophotometric analyses were carried out using a Shimadzu UV–VIS scanning spectrophotometer (Model UV-2101/1301 PC) (Bonakdarpour et al., 2011). The formation and degradation of RB5 metabolites was also followed by high-pressure liquid chromatography (Shimadzu – HPLC) analysis according to the procedure described in Bonakdarpour et al. (2011).