Short Communication

Decolourisation of a synthetic textile effluent using a bacterial consortium

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In the present study we examined the performance of a thermoalkalophilic bacterial consortium, where the predominant strain was *Bacillus* sp. SF, in the degradation of Reactive Black 5 (RB5). We used a reactor working in continuous mode and investigated the effects of pH, hydraulic retention time (HRT) and several added salts on colour and chemical oxygen demand (COD) reductions. For the chosen operational conditions (pH 9, 55°C and HRT of 12 h) the efficiencies achieved were 91.2 ± 0.8 % for colour removal and 81.2% for COD removal. The system tolerated, with no significant decrease in colour removal efficiency, 30 g/L Na_2SO_4, Na_2CO_3 or NaCl. The latter two salts, however, led to a reduction in COD removal of 30% and 50%, respectively. The system proved to be very effective in the decolourisation of C.I. RB5 under alkaline conditions and at a comparatively high temperature.

Azo dyes are easily reduced to colourless amines; however, in conventional biological treatment systems, like activated sludge, the prevailing aerobic conditions usually prevent complete reductive azo dye decolourisation [1–3]. Therefore, the treated water still needs some kind of tertiary treatment. Additional problems met in the treatment of wastewaters generated by dyeing with reactive azo dyes are the high temperature and pH values and the high salt content of reactive dye baths [4–7]. Therefore, in the last few years, intensive research has been performed in the azo dye biodegradation area, mainly with bacteria, either isolated or in consortia [2, 8–11], aiming at the pro-

duction of wastewaters complying with the applicable legislation. In Portugal, for instance, the limits for discharge of effluents from textile industries are as follows: pH between 5.5 and 9 (Sorensen scale), biochemical oxygen demand after 5 days <100 mg O_2/L, chemical oxygen demand (COD) <250 mg O_2/L, and colour not visible at a dilution of 1:40 [12]. These limits prevail over general regulations [13] for the discharge of residual waters.

In a biological system for wastewater remediation, the first consideration to be made relates to the choice of the biological material. In this respect, the use of bacterial consortia has several advantages. Firstly, they do not require sterile conditions, thus greatly reducing costs. Secondly, they are usually more stable towards changes in pH, temperature and feed composition, when compared with pure cultures [1]. Finally, there is a higher possibility of a complete mineralization of the dye since few strains have been found that can metabolise these types of compounds alone [8]. However, the operational parameters of a biological treatment system greatly influence its efficiency, and parameters such as hydraulic retention time

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Abbreviations: COD, chemical oxygen demand; **HRT**, hydraulic retention time; **RB5**, C.I. Reactive Black 5; **TSS**, total suspended solids; **TS**, total solids; **TVS**, total volatile solids; **VSS**, volatile suspended solids

(HRT), aeration level, temperature and pH must be optimised [8]. Also the microorganisms' ability to use nutrients such as acetate present in the effluents could help to reduce the cost and increase the applicability of a system in industry. Before a treatment system can be applied to industrial wastewater all the referred parameters must be studied and optimised.

The main goal of this study was to assess the influence of HRT, pH and the presence of common salts in reactive dye baths on the colour and COD removal efficiencies of a bacterial bioreactor applicable to the treatment of alkaline and coloured wastewaters at high temperature. A thermoalkalophilic bacterial consortium in which the predominant strain is *Bacillus* sp. SF was selected, based on the potential of its azoreductase to reduce azo dyes, as reported earlier [14, 15]. The reactor was operated at pH 9 and 55°C because reactive dyes are used under alkaline conditions at high temperatures. The chosen dye was C.I. Reactive Black 5 (RB5), one of the most widely used in cotton dyeing processes.

The bacterial consortium used in this work was isolated from a wastewater drain of a textile finishing company (Têxtil Alberto de Sousa, Guimarães, Portugal) based on its ability to degrade various dyestuffs at pH 9 and 60°C as previously described [14]. After a period of acclimation, the established bacterial consortium inside the reactor was found to be predominantly constituted by the *Bacillus* sp. SF strain [14, 15].

RB5 (min. dye content ~55%) was purchased from Aldrich and used without further purification. Inorganic media components, as well as glucose, were purchased from Merck. Peptone from casein and yeast extract were purchased from Difco. RB5 was used as a 0.4% solution in nutrient medium, which contained 2 g/L casein peptone, as carbon source, and 1 g/L yeast extract in a mineral salts solution with the following composition: 3.5 g/L KH_2PO_4, 7.5 g/L $Na_2HPO_4 \cdot 7H_2O$, 2.5 g/L $(NH_4)_2SO_4$, 4.5 g/L $MgSO_4 \cdot 7H_2O$, 0.2 g/L $MnSO_4 \cdot H_2O$ and 0.7 g/L $FeC_6H_5O_7 \cdot 5H_2O$. Trace elements were provided by the addition of 0.25% of the SL-6 trace element solution according to Pfennig [16]. All solutions were prepared with distilled water. To avoid precipitation of the salts during autoclave sterilisation, magnesium, manganese and iron salts were autoclaved separately and aseptically combined with the other components. Except where stated otherwise, medium pH was adjusted to 9.0 ± 0.2 with NaOH.

The bioreactor (scheme shown in Fig. 1) consisted of a cylindrical vessel 9 cm in diameter and 15 cm high (up to the conical top), filled with 0.65 L of a Leca® (expanded clay balls) carrier, produced and purchased from Dansk LecaA/S, Hinge/Randers, Denmark. The working volume was 0.45 L. A metal filter was fitted to the conical top to avoid the carrier washout. Except where stated otherwise, the reactor was operated at a flux of 37.5 mL/h (corresponding to an HRT of 12 h), under an aeration flux of

300 mL/min. The system also included an aeration pump Tagus 3000 and a feed pump Gilson model Minipulse2. The reactor temperature was maintained at 55 ± 2°C by a coil of flexible silicone tubing connected to a water bath at 60 ± 1°C. Whenever operating conditions were changed, the reactor was allowed to stabilize for four cycles (four times the HRT). Samples were collected daily from the reactor effluent.

The dye content in samples was calculated by absorbance measurements at 560 nm against a water blank. Samples were filtered with 0.20-μm membranes and diluted as required. Total suspended solids (TSS), total solids (TS) and total volatile solids (TVS) were determined by standard gravimetric methods [17]. The COD of dissolved matter was measured in filtered samples, using a HACH spectrophotometer model DR/2000, following the manufacturer's instructions for the HACH high range COD test (0–1500 ppm). All the presented results are the average of three independent determinations, except for COD analyses relating to the effect of salts, for which all collected samples were mixed to make a single composite sample for each salt tested. The reactor was operated for 380 days, during which the effects of HRT, pH and salts addition in the reactor performance were examined.

The influence of HRT on colour and COD removal is depicted in Fig. 2A. HRT colour removal efficiency increased from ~87% to 92% between 6 and 12 h, reaching ~95% at an HRT of 24 h. COD removal remained almost constant (~62%) between an HRT of 6 and 10 h, increasing to 81% at 12 h, and to 89% at 24 h. The low COD removal at low HRTs is possibly due to the incomplete consumption of medium nutrients, which account for the total COD amount. Despite the small difference in the values obtained for HRT <12 h, the existence of a dye adsorption step prior to biodegradation (that would limit the degradation rate until 12 h of HRT) may also be possible. After that the degradation rate would be controlled by the biodegradation mechanism. Considering the modestly efficient increase in colour and COD removal between
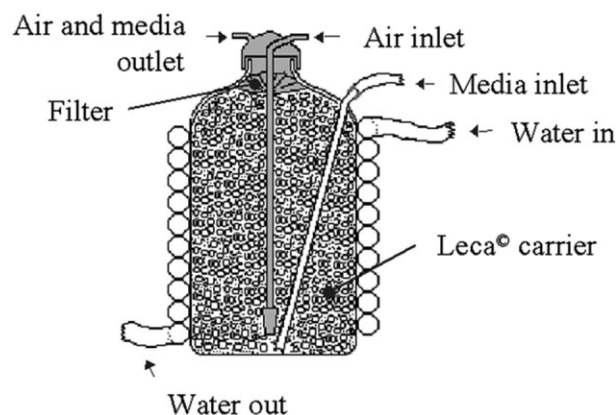

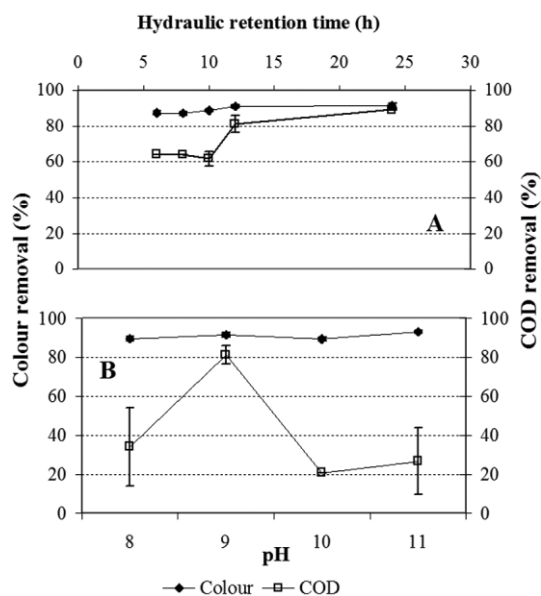**Figure 1.** Scheme of the lab scale reactor for continuous decolourisation.

Figure 2. (A) Effect of the HRT on colour and COD removal efficiencies at pH 9 and 55 ± 2°C (mean values and SD). (B) Effect of pH on colour and COD removal efficiencies at 12 h of HRT and 55 ± 2°C (mean values and SD).

Table 1. Effect of the presence of 30 g/L of NaCl, Na_2SO_4 and Na_2CO_3 on colour and COD removal efficiencies at 12 h of HRT at pH 9 and 55 ± 2°C

	Colour removal (%)	COD removal (%)
Control	91.2 ± 0.8	81.2
30 g/L NaCl	89.7 ± 1.7	31.0
30 g/L Na_2SO_4	92.4 ± 0.2	78.9
30 g/L Na_2CO_3	92.1 ± 0.4	51.6

was 0.54 ± 0.22 g/L. The lowest values were obtained for the operation at pH 11 (0.23 ± 0.11 g/L). This high average amount of solids in the effluent is not totally unexpected since the degradation achieved is high, meaning that the production of biomass is also high. Increasing the HRT, the amount of suspended solids in the effluent was increased as expected, although not very significantly (from 0.60 ± 0.03 g TSS/L at 8 h HRT to 0.68 ± 0.04 g TSS/L at 24 h HRT). The considerable variability in the observed results is, primarily, a consequence of factors related to the reactor's design, particularly clogging, which lead to oscillations in the aeration flux, biomass loss and development of anaerobic areas. Therefore, the application of this system in industry would require a more efficient aeration system and a sedimentation process in the effluent of the reactor since the amount of solids largely exceeds the allowed upper limit for discharge into an aquatic environment (in Portugal this is 60 mg/L [13]).

The biomass present inside the reactor, i.e., the biomass in suspension plus the biomass attached to the carrier, was determined as TVS. For determination of the attached biomass, four independent collections of carrier were performed. These samples were washed with water, dried at 85°C, weighted (W1), dried at 550°C, weighted (W2), washed again, dried at 85°C and weighted again (W3). The amount of TVS was determined by: TVS (mg VS/g carrier) = (W1–W2–W3)/W3. An average value of 16 ± 3.3 g TVS was obtained, from which approximately 4.5 ± 0.5% was in suspension. The carrier adsorbed an average of 20.6 ± 4.3 mg TVS/g carrier.

In summary, the major outcome from this study was the demonstration that this alkali-thermophilic bacterial consortium is also salt tolerant, achieving ~90% colour removal at pH 9.0 and 55°C, even in the presence of the tested salts. These are important advantages in the treatment of hot, high-salt wastewaters generated by dyeing baths. However, the production of VSS is still far too high for discharge and COD removal is negatively affected by salts, particularly by NaCl, which decreases this parameter from 80% to ~30%. Future work will require a tighter control of aeration inside the reactor (to minimise clogging, channelling and consequent development of anaerobic areas), optimisation of the ratio of carbon source/dye, the investigation of the mechanism(s) responsible for colour loss, the testing of other reactive dyes and the use of real wastewaters.

12 and 24 h, subsequent experiments were performed with an HRT of 12 h.

As the used consortium is alkalophilic, pH was varied between 8 and 11, and the results are shown in Fig. 2B. Within the tested range, dye removal did not significantly change; however, for COD removal, a maximum was observed at pH 9. Again since COD is primarily due to nutrient substrates, these results suggest that the consortium has an optimum metabolic activity at that pH.

The effects of 30 g/L NaCl, Na_2SO_4 and Na_2CO_3 were also investigated. In the dyeing processes, these salts are used mainly as auxiliaries to ensure maximum fixation of dye to the fibre [4, 18]. As shown in Table 1, the tested concentration did not significantly affect colour removal. In contrast, COD removal was negatively affected, particularly by NaCl. The same type of behaviour has been observed in an up-flow anaerobic sludge blanket reactor [18]. An explanation for this effect is that high salt concentrations (>1%) cause plasmolysis and/or loss of cell activity [19]. However, since efficiency in colour removal was not significantly decreased, the hypothesis of dye adsorption to biomass and/or carrier, as an initial step before biodegradation, cannot be discarded. It is also known that chloride anions increase COD [20]. This was verified in the increase value of COD in the fed of the reactor when NaCl was added, and could also have made a contribution to the decreased COD removal efficiency.

During reactor operation frequent analyses of the TSS in the collected samples (liquid phase) were performed. The mean value (± SD) during the operation of the reactor

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