Recycling food waste to clean water: the use of a biodigester's residual liquid inoculum (RLI) to decolourise textile azo dyes

A. C. Maganha de Almeida, J. Backhaus and C. R. Corso

ABSTRACT

A residual liquid inoculum (RLI) was used to decolourise solutions of Acid Yellow 25 (AY25) and Direct Violet 51 (DV51) azo dyes. The RLI was obtained through anaerobic digestion of food waste from a university restaurant. The concentration of bacteria in the RLI was 8.45×10^7 CFU mL^{-1}. Dye solutions (50 $\mu g\ mL^{-1}$) were inoculated with the RLI (20% v/v) and incubated at room temperature. The decolourisation studies took place at microaerophilic and in-batch conditions and at pH = 2.50. Initially, the dyes were taken up from solution by biosorption; maximum colour removal was achieved after 3 hours of incubation, with 88.66% for AY25 and 77.65% of DV51. At prolonged incubation times (3–96 hours) decolourisation was mainly attributed to biodegradation of the azo solutions, with breakage of the azo bond, as detected by UV-VIS spectroscopy and Fourier transform infrared (FT-IR) analysis. Analysis of UV-VIS absorption rates of dyes showed, however, that AY25 was more readily biodegradable whereas DV51 was more recalcitrant to the action of the RLI.

Key words | Acid Yellow 25, anaerobic biodigester, biodegradation, biosorption, Direct Violet 51, microbial consortium

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INTRODUCTION

The fashion industry, with its demand for new colours and fabrics, fuels production of synthetic dyes and has resulted in the textile industry becoming one of the greatest polluters of water sources. Azo dyes (-N = N-) (Anliker 1977; Abraham *et al.* 2003) – the predominant class of molecules used in textile dyeing (Zollinger 2003; Santos & Corso 2014) – have an estimated yearly production of $\sim 9 \times 10^6$ tons (Rawat *et al.* 2016). During the textile colouring processing, approximately $\sim 7 \times 10^5$ tons of azo dyes (15% of the yearly production) does not bind to fibres and so are lost in textile effluents (Delee *et al.* 1998; Liao *et al.* 2013). Rawat *et al.* (2016) presents a more drastic statistic with over 4.5×10^6 tons of azo dyes and their by-products being lost yearly in textile effluents.

Azo dyes are visible at concentrations as low as 1 mg L^{-1}. Textile effluents have concentrations 10 to 200 times higher than that (Guaratini & Zanoni 2000). At these levels they obstruct light penetration and lower oxygen transfer (Alves de Lima *et al.* 2007; Pandey *et al.* 2007; Mitter & Corso 2013; Guari *et al.* 2015), compromising both aesthetics and the ecological balance of water bodies (Corso & de Almeida 2009; Guari *et al.* 2015). Azo dyes and, in particular, their by-products may have carcinogenic and genotoxic effects for humans and aquatic biota (Balakrishnan *et al.* 2016). Drinking water sources contaminated by levels of textile effluents at 3% have been shown to present high levels of mutagenicity and carcinogenicity, even after the water had undergone treatment by local authorities (Alves de Lima *et al.* 2007).

Amongst the methods currently used to treat textile effluents are physico-chemical technologies, such as membrane filtration, coagulation, flocculation and adsorption. The downsides of these methods include cost and the generation of residual waste/sludge which requires further management (Robinson *et al.* 2001; Mitter & Corso 2013). Advanced oxidative processes (AOPs) minimise the residual waste and are effective at bench-scale (Alaton *et al.* 2002; Agorku *et al.* 2015); their cost and deployment at large-scale, however, are still prohibitive (Robinson *et al.* 2001; Jadhav *et al.* 2016).

The discharge of untreated or poorly treated textile effluents into water sources must be avoided. In the

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search for lower production costs, textile industries have moved their production sites to developing and underdeveloped countries. In such places, environmental controls and practices are weaker and so are less likely to prevent discharge of coloured effluents into surface waters. Many industries in the region of Americana and Rio Claro (São Paulo State, Brazil), for example, at risk of being charged by the local environmental agency, bleach their effluents to ensure it passes compliance tests (Contato & Corso 1996). A severe downside of this practice is that although colour has been removed from the effluent, potentially more toxic by-products prone to being carcinogenic and having high toxicity levels may have been potentially produced and discharged into the environment. In order to compel the textile industry to treat textile effluents to higher quality standards, more affordable, on-site and easily deployed methods of treating textile effluents need to be developed. These may help to persuade decision-makers in the industry to opt to treat or reuse rinse-water onsite in a 'closed-loop' or 'zero-pollution' manufacturing chain, thus avoiding discharge of textile effluents into the environment (Sarkis 2001).

Bioremediation is increasingly being used as an alternative low-input, cost-effective and environmentally safe way to treat textile effluents (Delee *et al.* 1998; Kunz *et al.* 2002; Rawat *et al.* 2016). Biodegradation and biosorption are bioremediation processes which are typically used for the decolourisation of azo dyes. They are a result of cleavage of dye chromophore groups into simpler molecules and biosorption of the dye molecules by the microbial biomass, respectively (Kalme *et al.* 2007). Individual species of bacteria (Pandey *et al.* 2007; Cerboneschi *et al.* 2015), yeast (Jadhav & Govindwar 2006; Jadhav *et al.* 2007; Vitor & Corso 2008), white-rot fungi (Pointing 2001) and wood-degrading mushrooms (Cohen *et al.* 2002) have been proven capable of decolourising water contaminated by azo dyes.

The use of microbial consortia to decolourise azo dyes has advantages over the use of single species alone (Mikesková *et al.* 2012). Microorganisms in a microbial consortium act synergistically (Sin *et al.* 2016): while one microbial species may attack a certain group of the dye molecules, the other may help to mineralise the rest of the molecules thus decreasing the chances of harmful by-products being formed. A microbial consortium does not require sterile conditions, and is robust to changes in the environment, such as pH, temperature, and feed conditions. Accordingly, the microbial consortium is more likely to endure unfavourable environmental conditions in textile effluents, such as high chemical oxygen demand (COD) and salinity, and to achieve success in bioremediation (Jadhav *et al.* 2016).

Biodigesters are one of the most promising, sustainable, and versatile biotechnologies for recycling organic waste, through anaerobic digestion, for producing biogas (Cestonaro do Amaral *et al.* 2016), and for recovering nutrients for agro-industry (Borja & Banks 1994; dos Santos Reis *et al.* 2016; Shutts *et al.* 2016). Biodigesters reduce environmental impacts of organic waste and decrease organic load on landfills. During the process of anaerobic digestion, biodigesters produces a residual liquid, also called 'effluent' or 'digested bioslurry'. This by-product is rich in nutrients, such as phosphorus and nitrogen, and is an invaluable source of fertilisers (Rodríguez *et al.* 2009; Roy 2017). Residual liquid from biodigesters also contains a pool of versatile microbial consortia (Moraes & Paula Júnior 2004), with promising and unexplored abilities to bioremediate polluted soil and water environments.

Our study aimed to explore this niche by investigating the biodegradation and biosorption of Direct Violet 51 and Acid Yellow 25 by a residual liquid inoculum (RLI) in aqueous solution. The RLI was produced by the anaerobic digestion of food waste using a pilot-scale biodigester. The objectives were: (i) to produce an inexpensive inoculum by recycling food waste through a biodigester and (ii) to determine its biodegradation and biosorption abilities. The prevalence of biodegradation or biosorption was calculated by using absorbance ratios calculated from the UV-VIS spectra of decolourised supernatants (Glenn & Gold 1983; Santos & Corso 2014). The biodegradation route of the azo dyes and by-products formed were investigated using Fourier transform infrared (FT-IR) analysis.

MATERIALS AND METHODS

Chemicals and media

Chemicals were obtained from Sigma-Aldrich, Reidel-de Haën, BDH Chemicals, and Fluka Analytical. All chemicals used were analytical grade. All media were obtained from Oxoid unless otherwise stated. All media were sterilised by autoclaving at 121 °C for 15 min.

Azo dyes and preparation of azo dyes solutions

The azo dyes Acid Yellow 25 (C.I. 18835-Aldrich 20,196-0) and Direct Violet 51 (C.I. 27905-Aldrich 21,238-5) were obtained from Imperial Chemistry Industries, a dye

manufacturing unit in Rio Claro, São Paulo, Brazil. Direct Violet 51 and Acid Yellow 25 are used in the leather industry (Guillén *et al.* 2012) and in wool dyeing, respectively (Periolatto *et al.* 2011). The stock solutions of Acid Yellow 25 and Direct Violet 51 (1,000 μg mL^{-1}) were prepared by diluting dye powder in distilled water at pH 2.50 (adjusted with H_2SO_4 at concentrations ranging from 0.01 M to 1.0 M). The pH value of 2.50 was chosen based on Vitor & Corso (2008) and Mitter & Corso (2013), as these authors have determined that dye removal is more efficient under acidic conditions.

Production of RLI in biodigester

The RLI was obtained using a laboratory-scale biodigester made of stainless steel with a total capacity of 15 L. The laboratory-scale batch biodigester (Figure 1(a–d)) was fed with food waste (10 L) collected from the student canteen of Mannheim University of Applied Sciences. The waste consisted of raw and cooked food waste, such as vegetables, meat and fruit. Prior to being used as feed, the waste was blended using a domestic blender at maximum speed for 1 min (dos Santos Reis *et al.* 2016). The biodigester was started

Figure 1 | Set up of biodigester: (a) stainless steel container and PVC tube + metal sieve filtration unit + PVC lid, (b) stainless steel 15 L container, (c) full view of filtration unit, (d) biodigester top view.

up by inoculating 0.1 L of an RLI, which had been extracted from a active biodigester (de Almeida *et al.* 2006). The batch biodigester was then incubated at 25 °C for 30 days (mesophilic conditions). After this period, the fermented bulk presented three layers: an upper fat layer, an intermediate liquid phase, and a solid sediment layer. The liquid phase was extracted using a filtration system made from a 50 mL syringe and a PVC tube and stainless-steel sieve (of a type used to prepare tea) (Figure 2(a)). The liquid extracted was then centrifuged at $896 \times g$ (4,000 rpm). After centrifugation, three layers were formed again, and the intermediate liquid (the RLI) was extracted from the centrifuge tube using sterile 3 mL Pasteur pipettes. The pure RLI extract (Figure 2(b)) was preserved at 4 °C for a maximum period of 30 days before decolourisation studies.

RLI analysis

Aliquots of 1 mL of the RLI were serial diluted in 9 mL of saline solution (0.85% w/v). Dilutions were plated by pour-plate technique in plate count agar (PCA) and potato dextrose agar (PDA). Plates were made in triplicate and incubated at 30 °C (PCA) for 48 hours and at 22 °C (PDA) for 5 days.

Decolourisation studies

Samples were prepared in 5 mL test tubes with 0.250 mL of dye stock solution, 0.125 mL RLI and 4.625 mL of distilled water at pH 2.50 (adjusted with H_2SO_4 at concentrations ranging from 0.01 M to 1.0 M). After inoculation, test tubes were capped and incubated at 25 °C ± 1 °C for different contact times. Following the period of contact between the RLI biomass and the dyes, the solutions were centrifuged at $896 \times g$ (4,000 rpm) for 10 min and the supernatant were analysed by UV-VIS and FT-IR methods.

UV-VIS analysis: percentage of colour removal

UV-VIS analyses were performed in samples of supernatant, using 5 mm quartz cuvettes which were scanned between 800 and 190 nm using a HP8453 UV-VIS. Absorbance of dye control (50 μg mL^{-1}), RLI control + distilled water (0.125 mL/0.485 mL) and treatment (dye + RLI + distilled water diluted as per concentration above) were evaluated. The percentage of colour removal (%) was obtained from the correlation described by Cripps *et al.* (1990) in Equation (1):

$$\frac{A_0 - A_t}{A_0} \times 100 \quad (1)$$

where:

A_0 = initial Abs λ^{Max}
A_t = Abs λ^{Max} at time t

UV-VIS analysis: calculation of absorption ratios

Predominance of biosorption or biodegradation was studied using the method of 'absorbance ratios' (Glenn & Gold

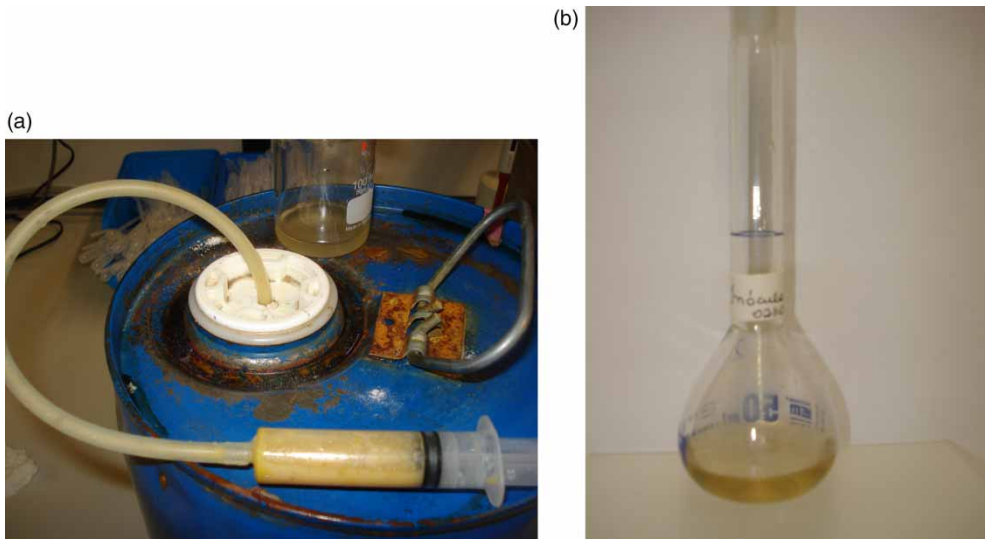

Figure 2 | (a) Extraction of intermediate liquid layer using (a) 20 mL syringe and PVC tube. (b) RLI ready to use, after syringe extraction, centrifugation and filtration.

1983). Absorbance ratios are determined by Equation (2):

$$\frac{\text{Abs}\,\lambda^{\text{Max}}}{\text{Abs}\,\lambda^{\text{Max}/2}} \quad (2)$$

where:

Abs λ^{Max} = highest absorbance at wavelength λ
Abs $\lambda^{\text{Max}/2}$ = absorbance corresponding to half λ_{Max}

FT-IR analysis

Supernatant of samples (as well as controls of dye and RLI) were placed in crucibles and dried for 48 hours at 105° ± 1 °C. The samples were then removed and placed in a desiccator for 24 hours. For pellet preparation, a dried sample (approximately 1 mg) was ground thoroughly with 149 mg of KBr and submitted to compression at 40 kN for approximately 5 min. The pellet (translucent appearance, approximate diameter of 13 mm and thickness of 2 mm) was immediately placed in a Bruker Vector 22 spectrophotometer (400–4,000 cm^{-1}, 16 scans, resolution 4 cm^{-1}). Spectra were smoothed and presented in terms of absorbance.

Data analysis

All experiments were repeated three times. Samples were prepared in triplicate. Averaged spectra were used for analysis. Variation of absorbance between spectra of treated samples was never more than 10%.

RESULTS

Characterisation of RLI

After 30 days of incubation the RLI presented pH of 3.43 and an acetic smell. There were 8.45×10^7 CFU mL^{-1} bacteria present on the PCA plates. Growth on the PDA plates was not observed. The RLI did not present absorption in the visible range therefore allowing observation of the treated dyes without interference of the VIS region.

UV-VIS analysis: percentage of colour removal

The percentages of colour removal of Acid Yellow 25 (AY25) and Direct Violet 51 (DV51) from solution by RLI were calculated (Equation (1)) and are shown in Table 1 (Cripps *et al.* 1990). Decolourisation of azo dyes was greater than 75% however removal of DV51 was greater than of AY25.

Table 1 | Decolourisation of azo dyes in solution at pH 2.50 after 3 hours of contact with RLI biomass

Dye	Acid Yellow 25	Direct Violet 51
% Decolourisation after 3 hours	75.65	88.66

UV-VIS analysis: calculation of absorption ratios

UV-VIS spectra of AY25 (Figure 3) and DV51 (Figure 4) show decolourisation by RLI action occurred predominately

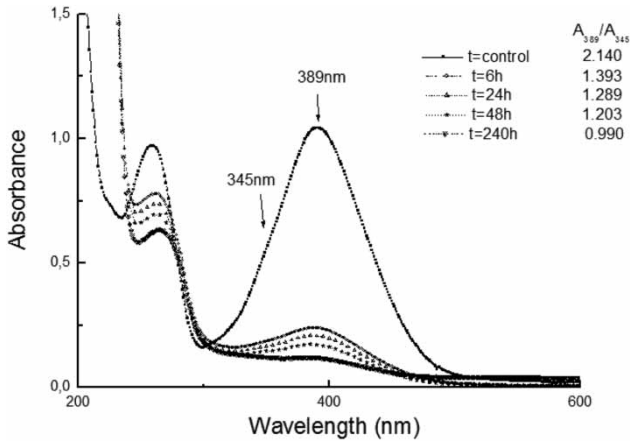

Figure 3 | Absorption spectra of Acid Yellow 25 dye solution at pH 2.50 after different contact times with the RLI at 25 ± 1 °C, with scans performed at 6, 24, 48 and 240 hours. Values of absorption ratios (Abs λ_{389}/Abs λ_{345}) are displayed for each contact time in the legend (top right).

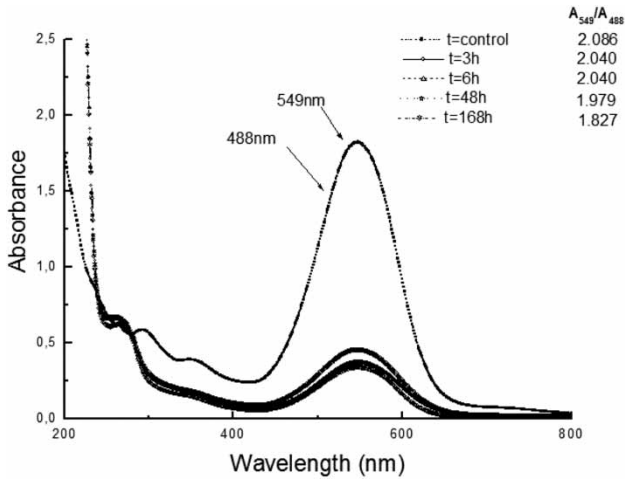

Figure 4 | Absorption spectra of Direct Violet 51 dye in solution at pH 2.50 after different contact times with the RLI at 25 ± 1 °C, with scans performed at 3, 6, 48 and 168 hours. Values of absorption ratios (Abs λ_{649}/Abs λ_{488}) are displayed for each contact time in the legend (top right).

at shorter incubation times. The analysis of UV-VIS absorption spectra (Equation (2)) of treated and untreated samples of AY25 showed that absorbance ratios decreased with time (Figure 3 inset). This indicates the chromophore groups of the AY25 molecules were not removed at a constant rate. This trend strongly indicates biodegradation of the dye molecules took place during decolourisation. In comparison, the values of absorbance ratios of DV51 (Figure 4 inset) decreased less with time. This trend relates to the chromophore groups of DV51 being removed at relatively more constant rates, indicating a predominance of biosorption.

FT-IR analysis of dyes

When the FT-IR spectra of controls of dye solutions were compared with samples (Figure 5(a) and 5(b)) – immediately after coming into contact with the RLI – no significant changes of bands were noticed. Therefore, bands emerging during treatment were connected to metabolites of dye biodegradation. In the control of AY25 peaks at 3,411 cm^{-1} and 1,340 cm^{-1} represented N–H stretching of aromatic amines (Kalyani *et al.* 2009; Almeida & Corso 2014). The sulfoxi-nature of AY25 was noted by a band at 1,376 cm^{-1}, characterising axial asymmetric stretching of the S = O group (Stuart & Ando 1997). Typically, sharp bands at 2,943 cm^{-1}, 752 cm^{-1} and 620 cm^{-1} were linked to out-of-plane bending vibrations of methyl aromatic groups. The azo bond was detected by weak bands at 1,406 and 1,547 cm^{-1} (Stuart & Ando 1997; Kalme *et al.* 2007; Parshetti *et al.* 2007).

Formation of primary and secondary aromatic amines, with peaks at 3,405 cm^{-1}, 2,930 cm^{-1} (N–H stretching) and 1,534 cm^{-1} (N–H bending), were detected at longer exposure times (Günzler & Gremlich 2002; Almeida & Corso 2014). Production of benzo-sulfonic groups were linked to 1,123 cm^{-1} and 1,660 cm^{-1}; a band at 1,025 cm^{-1} was linked to stretching of S = O (sulfoxides) (Stuart & Ando 1997). Aromaticity of sub-products was increased by generation of non-coloured benzene groups represented by peaks at 752 cm^{-1}, 619 cm^{-1} and 703 cm^{-1}. Aliphatic C–H bending was shown by peaks at 1,443 cm^{-1} and 1,416 cm^{-1} potentially linked to generation of nitroalkanes as a result of further degradation of amines (Jadhav *et al.* 2007; Kalme *et al.* 2007). A proposed pathway to possible metabolites formed during biodegradation of AY25 is shown in Figure 6.

FT-IR analysis of controls of DV 51 at pH 2.50 showed a band at 2,855 cm^{-1} which was linked to C–H aromatic stretching (Stuart & Ando 1997). Strong peaks at 883 cm^{-1}, 854 cm^{-1} and 642 cm^{-1} were attributed to out-of-plane bending of the C–H of aromatic rings. Bands at 3,427 cm^{-1} and at 2,922 cm^{-1} symmetric and asymmetric stretching of N–H, respectively (Kalyani *et al.* 2009) can be

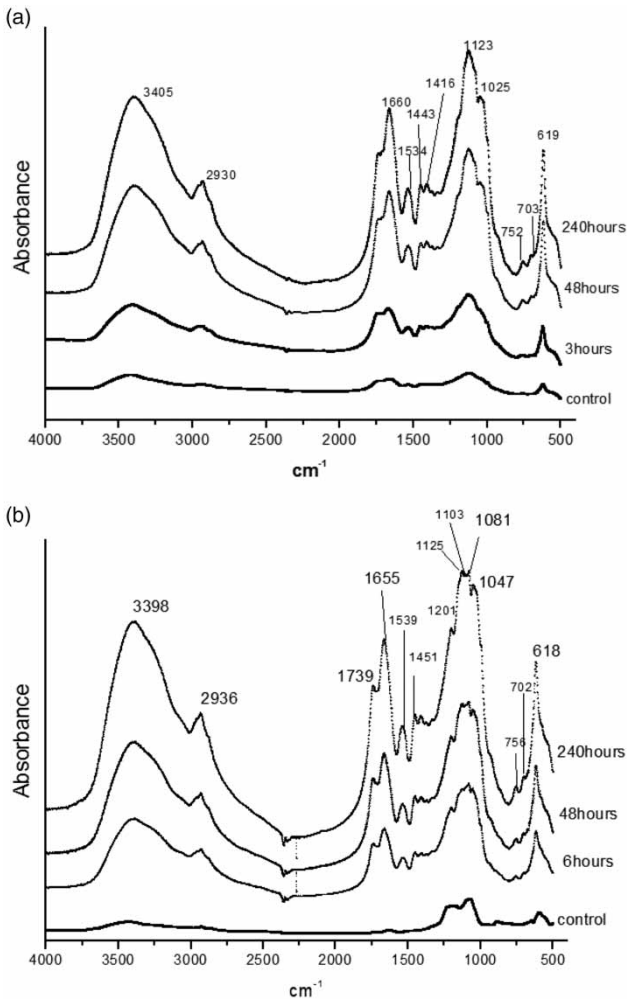

Figure 5 | FT-IR spectra of (a) AY25 and (b) DV51 controls and after various contact times with the RLI microbial consortium at pH 2.50.

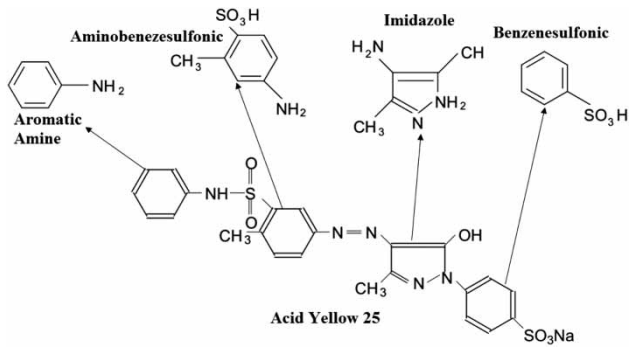

Figure 6 | Proposed pathways of biodegradation of AY25 by the RLI.

linked to naftol or other aromatic groups. Azo dye was detected at 1,633 cm^{-1} by stretching vibrations of N = N (Telke *et al.* 2009). A peak at 1,172 cm^{-1} was attributed to sulfonated groups ($R–SO_2^{3-}$) in the control dye (Li *et al.* 2009; Pinggui *et al.* 2009).

During biodegradation of DV51 at pH 2.50, the peak at 2,936 cm^{-1} indicated generation of aromatic amines. Nitro compounds –C–NO_2– were also detected at 1,739 cm^{-1} and 1,539 cm^{-1} representing carbonyl stretching and N–H bending, respectively (Stuart & Ando 1997). Peaks between 1,047 cm^{-1} and 1,201 cm^{-1} were related to benzosulfonic groups, matching the presence of two sulfonic groups in the parental dye molecule. Besides that, stretching of S = O (sulfoxides) would be represented by 1,047 cm^{-1}. Naftol and free primary amines were associated with peaks at 3,398 cm^{-1}, 2,936 cm^{-1} and 1,201 cm^{-1}. Aromaticity of by-products increased as indicated by sharp bands at 756 cm^{-1}, 702 cm^{-1} and 618 cm^{-1}. The band 1,081 cm^{-1} was present on both control and treatment, and was linked to the aromatic ether. A proposed pathway to possible metabolites formed during biodegradation of DV51 is shown in Figure 7.

DISCUSSION

The objective of the present study was to investigate the ability of an RLI, recycled from food waste, to decolourise two azo dyes in aqueous solution. The RLI was – inexpensively – produced through anaerobic digestion of food waste. Typically, food waste is disposed of in landfill or used to feed livestock (Lin *et al.* 2013). Here we suggest an alternative use for food waste by recycling through anaerobic digestion. During the process of anaerobic digestion, three commodities may be obtained: compost, biogas and RLI. In this study, we focused on the production and novel use of the third product – RLI – to bioremediate polluted environments.

As soon as it was extracted from the intermediate layer of the bulk fermenting in the in-batch biodigester, the RLI presented low pH (3.43) and, also, an acetic smell was noted. These observations evidenced that anaerobic digestion had achieved acidogenesis/acetogenesis phase. When the aim of anaerobic digestion is to produce biogas (dos Santos Reis *et al.* 2016), the acidified bulk should be buffered to adjust pH between 6.5–8.0, otherwise methanogenic bacteria will be inhibited (Mara & Horan 2003). In the present work, the aim was to produce the RLI, with the least interference possible to the in-batch biodigester and, therefore, pH adjustment of the bulk was not carried out. Typically, biogas plants run at temperatures between 30 and 37 °C. In the present work however, to avoid heating-related costs and to simplify the operation of the biodigester, anaerobic digestion of food waste was operated at 25 °C (mesophilic conditions).

The RLI decolourised AY25 and DV51 at rates comparable to rates achieved by microbial populations isolated from textile effluents and which had been pre-adapted to

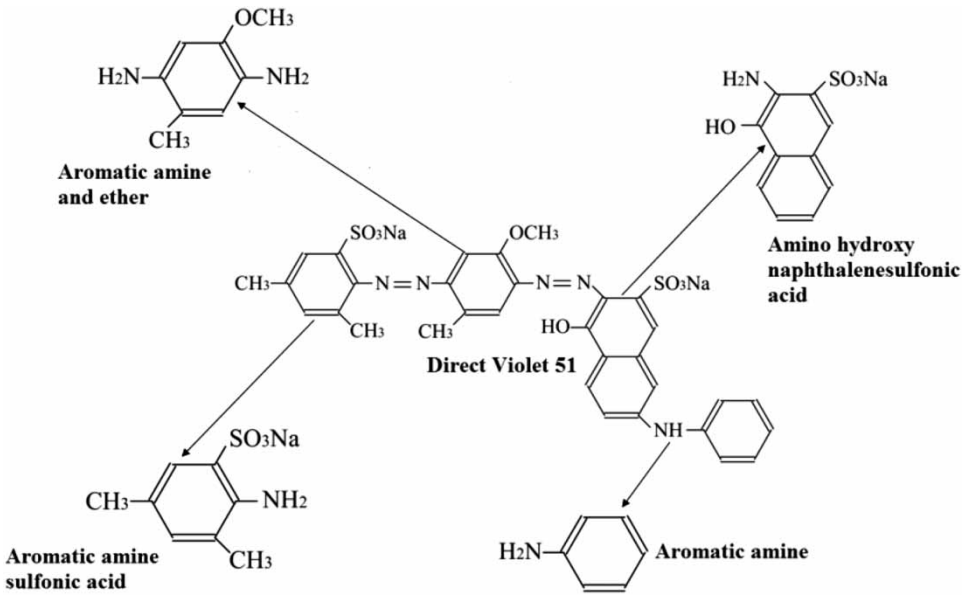

Figure 7 | Proposed pathways of biodegradation of DV51 by the RLI.

exposure to azo dyes (Jadhav *et al.* 2010; Phugare *et al.* 2011a, 2011b; Lade *et al.* 2012; Lade *et al.* 2016). The results of the UV-VIS spectroscopy analysis demonstrated a high percentage of colour removal for AY25 at pH 2.50. They also showed that the removal of DV51 from solution was superior. This may be explained by the fact DV51 has two negatively charged sulfonic groups, while AY25 has only one. These negative charges would have interacted and biosorbed more onto the microbial biomass. The two sulfonic groups in DV51, however, also increased its recalcitrance. Experiments in our laboratory tested the efficiency of RLI to decolourise AY25 and DV51 at pH 4.50 and pH 6.50 (data not shown) but the maximum colour removal was achieved at the lower pH of 2.50. Superior decolourisation at pH 2.50 was an expected result and is likely to have been a consequence of the interaction of sulfonic groups of the dye molecules with groups of the biomass which became protonated in acidic conditions (O'Mahony *et al.* 2002).

In the present work, decolourisation studies took place in capped test tubes, at static conditions, consequently with restricted oxygen availability to the RLI's microbial consortium. The reduced availability of oxygen may have facilitated reduction of AY25 and DV51 by the RLI. As empirically demonstrated by previous authors (Kalme *et al.* 2007), and as postulated by Stolz (2001), oxygen may compete with azo dye molecules for the reduced electron carriers, thus impairing dye reduction. Also, the rich nutrient composition of the RLI was expected to provide electron donors in abundance to the decolourising enzymes of the RLI consortium, such as azo-reductases. Electron donors are known to influence rates of azo dye reduction (Stolz 2001; Kalme *et al.* 2007) and may have acted synergistically to azo-reductases and/or other enzymes of the RLI, facilitating dye reduction.

Furthermore, a factor which could also have accounted for the successful removal of colour by the RLI is the aggregation of the consortium (Shabbir *et al.* 2017) and the presence of extracellular polymeric substances (EPS). Generally, under unfavourable and/or stressful environmental conditions, microbial cells produce EPS and naturally aggregate. This process is also known to severely impact chemical and physical disinfection (de Almeida & Quilty 2016). Under the acidic conditions of the fermented bulk, microbial cells were exposed to acidic pH and potential environmental stress. This condition could have easily triggered aggregation and production of EPS cementing matrix. Upon exposure to the azo dyes, microbial cells of the RLI could have aggregated further. Planktonic cells of *Pseudomonas putida* CP1 were shown to aggregate as soon as they were exposed to Rose Bengal solutions at 50 $\mu g\ mL^{-1}$ (de Almeida 2013). Aggregated cells of the RLI, united and protected by the cementing EPS matrix, would have interacted more readily with the dye molecules. The immobilisation of the dyes onto the aggregated biomass could in turn have enhanced rates of biodegradation and biosorption.

The measurement of absorbance ratios is an analysis method described by Glenn and Gold (Glenn & Gold 1983). This method has been used and validated by our research group in several studies (Vitor & Corso 2008; Santos & Corso 2014). The steadiness of absorbance ratio values represented a proportional decrease in absorbance at all wavelengths, which is expected from homogenous colour removal taking place by biosorption (Vitor & Corso 2008). Breakage of chromophore bonds may cause high variation of absorbance ratios as a consequence of biodegradation. The analysis of absorbance ratios of AY25 and DV51 helped in inferring whether decolourisation was predominantly caused by biosorption or biodegradation. The variation of absorbance ratios (A_{549}/A_{488}) of DV51 was less than for AY25 (A_{389}/A_{345}). In this way, UV-VIS analysis helped to demonstrated further that DV51 was more resistant to biodegradation and more readily biosorbed onto RLI's biomass than AY25.

The profile of FT-IR analysis showed partial mineralisation of the azo dyes. The major differences observed between control and treatments were breakage of the azo bond with detection of aromatic primary amines for both dyes (AY25 at 3,405 cm^{-1}, 2,930 cm^{-1} 1,534 cm^{-1} and DV51 at 3,398 cm^{-1}, 2,936 cm^{-1} and 1,201 cm^{-1}). Anaerobic decolourisation of azo dyes has one main drawback, which is the generation of uncoloured and persistent aromatic amines (Delee *et al.* 1998; Abraham *et al.* 2003). This is a concern from the toxicity perspective of treated textile effluents. Several studies have shown that efforts to mineralise aromatic amines may be achieved under aerobic conditions and should continue to be investigated d (Delee *et al.* 1998; Pandey *et al.* 2007; Jadhav *et al.* 2016).

Another major trend observed was the detection of benzosulfonic groups (AY25 at 1,660 cm^{-1} and DV51 at 1,047 cm^{-1} and 1,201 cm^{-1}). The trend of breakage of the azo dye and formation of benzosulfonic groups follows the biodegradation path observed by previous studies with Direct Blue 71 treated with *Aspergillus oryzae* and *Phanerochaete chrysosporium* (Santos & Corso 2014). There was also evidence of further mineralisation of the aromatic amines in AY25 with formation of nitroalkanes as a result of further degradation of amines (1,443 cm^{-1} and 1,416 cm^{-1}).

We demonstrated an inexpensive way to recycle food waste and produce microbial biomass capable of decolourising azo dyes. Research efforts should work towards a closed-loop approach to production where food waste is minimised or, if that is not possible, recycled to produce energy and/or raw materials with aggregated value. The procedure adopted to extract the RLI from the acidic digested bulk (by filtration and centrifugation) may have excluded part of the microbial diversity, and particularly microorganisms found aggregated or embedded in solid parts of the bulk. In the interests of the time available to conduct the present research, we focused on the environmental application of the RLI; we suggest future studies could focus on developing protocols to extract other parts of the microbial population from the solid bulk, and also explore the bioremediative potential of RLI from other phases of anaerobic digestion.

Future research should also focus on further mineralisation of the sub-products formed, in particular on aromatic amines. An interesting approach would be to optimise the RLI consortium with the purpose of using it for bioaugumentation in anaerobic/aerobic treatment in pilot plants. As suggested previously (Mikesková *et al.* 2012), use of chemometry methods, such as the response surface methodology, could help in the optimisation of the RLI microbial consortia through statistical analysis to achieve this aim. Since we performed the anaerobic digestion at a mesophilic temperature (25 °C), future studies may consider addressing the effects of higher temperature ranges on both the biodigester's performance and rates of decolourisation studies.

Additionally, AOPs, especially those driven by renewable sources such as sunlight, are also a promising technology that could be allied to the aerobic mineralisation of the by-products of azo-dye reduction. AOPs, such as ozonation, have been shown to produce biodegradable by-products from textile dyes and could also be used as an option for pre-biological treatment (Bilińska *et al.* 2016). We close our study with a suggestion to environmental entrepreneurs and stakeholders involved with the creation and implementations of R&D solutions for textile wastewaters and food-waste reuse: anaerobic biodigestion of food-waste can be explored to produce the RLI, a valuable commodity that cleans coloured wastewaters.

CONCLUSIONS

Azo dyes can be decolourised by an RLI produced from anaerobic digestion of food waste. Biodegradation was the predominant process in the colour removal of AY25, whereas for DV51 it was biosorption. The presence of two sulfonic groups in DV51 potentially facilitated attachment of the dye molecules to the RLI biomass; however, it also increased recalcitrance by hindering enzymatic attack. As shown by FT-IR, decolourisation of both dyes was accompanied by the formation of simpler by-products and the disappearance of the azo bond. We encourage future investigations with RLI to bioremediate polluted waters.

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