Biodegradation of textile wastewater: enhancement of biodegradability via the addition of co-substrates followed by phytotoxicity analysis of the effluent

María Belén Ceretta, Ignacio Durruty, Ana Micaela Ferro Orozco, Jorge Froilán González and Erika Alejandra Wolski

ABSTRACT

This work reports on the biodegradation of textile wastewater by three alternative microbial treatments. A bacterial consortium, isolated from a dyeing factory, showed significant efficacy in decolourizing wastewater (77.6 ± 3.0%); the decolourization rate was 5.80 ± 0.31 mg of azo dye·L^{-1}·h^{-1}, without the addition of an ancillary carbon source (W). The degradation was 52% (measured as COD removal) and the products of the treatment showed low biodegradability ($COD/BOD_5 = 4.2$). When glucose was added to the wastewater, (W + G): the decolourization efficiency increased to 87.24 ± 2.5% and the decolourization rate significantly improved (25.67 ± 3.62 mg·L^{-1}·h^{-1}), although the COD removal efficiency was only 44%. Finally, the addition of starch (W + S) showed both a similar decolourization rate and efficiency to the W treatment, but a higher COD removal efficiency (72%). In addition, the biodegradability of the treated wastewater was considerably improved ($COD/BOD_5 = 1.2$) when starch was present. The toxicity of the degradation products was tested on *Lactuca sativa* seeds. In all treatments, toxicity was reduced with respect to the untreated wastewater. The W + S treatment gave the best performance.

Key words | bacterial consortium, biodegradation kinetics, industrial starch, toxicity, textile wastewater

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INTRODUCTION

Textile industries produce wastewater that contains several kinds of chemicals such as dispersants, mordant agents, acids, carriers and various dyes. To dye 1 kg of cotton, 0.6–0.8 kg NaCl, 30–60 g dyeing materials and 70–150 L of water is necessary (Babu *et al.* 2007). The wastewater produced by the textile dyeing and finishing processes has unbound reactive dyes, high salt content and dyeing additives (Babu *et al.* 2007; Sen *et al.* 2016). Each day, the textile factories release millions of litres of untreated wastewater into public sewers; this is eventually discharged into rivers or seas. This pollution modifies the pH, and increases the biochemical oxygen demand (BOD) and the chemical oxygen demand (COD), causing intense coloration of the receiving water. The colour interferes with penetration of sunlight, photosynthesis, inhibits the growth of aquatic biota and interferes with the solubility of gases in natural water bodies (O'Neill *et al.* 1999). In addition, during the dyeing process about 50% of the dye loses its affinity towards the fabric and remains with the spent dye bath effluent and cannot be re-used (Watanapokasin *et al.* 2009). Furthermore, the reduction of several azo dyes causes the formation of aromatic amines, which have been reported to be both toxic and carcinogenic (Novotny *et al.* 2006). For this reason, it is important to analyze the product of such processes for toxicity.

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In Mar del Plata, 32% of the industrial wastewater belongs to the textile industry. In addition, pollution has been detected as a consequence of the illegal dumping of dyes into pluvial collectors from a dyeing workshop (Concejo Deliberante Mar del Plata-Batán 2007; www.0223.com.ar 2007). This type of effluent is a problem of great concern for public health.

Several physico-chemical methods have been applied to the treatment of textile wastewater, but these methods have many limitations due to high cost, low efficiency and secondary pollution problems (Singh & Singh 2017). In recent years, biological processes for dye degradation and wastewater reutilization have been developed, including aerobic and anaerobic treatments with bacteria (Chengalroyen & Dabbs 2013; Jadhav *et al.* 2016) and aerobic treatments with fungi (Durruty *et al.* 2015; Sen *et al.* 2016). Work on bacterial degradation of dyes was started in the 1970s with a report on *Bacillus subtilis* (Horitsu *et al.* 1977); since then, several experiments have been carried out studying the biological degradation of azo dyes by numerous other bacteria (Jadhav *et al.* 2016; Chen *et al.* 2018; Srinivasan & Sadasivam 2018). However, only a few of these works reported results concerning the treatment of wastewater collected directly from the factory (Amaral *et al.* 2014; Sathian *et al.* 2014; Vijayalakshmidevi & Muthukumar 2015; Balapure *et al.* 2016; Kurade *et al.* 2017) treated with a mixed culture isolated from the same industrial facility (Amaral *et al.* 2014). In most of the studies, a number of bacterial or fungal species have been isolated from places contaminated with textile wastewater; then, these specific bacterial species were mixed together and used to treat the wastewater. For example, Vijayalakshmidevi & Muthukumar (2015) described the biodegradation of the textile effluent using a mixed culture of *Ochrobactrum* sp., *Providencia vermicola* and *Pseudomonas aeruginosa*, which were previously acclimated to the textile effluent. In turn, Balapure *et al.* (2016) used a mixed culture (BDN) composed of eight different bacterial strains previously isolated and enriched in a culture supplemented with Reactive Blue 160. Kurade *et al.* (2017) reported the decolourization of textile effluent by a bacterial-yeast consortium.

Usually, individual bacterial strains are not able to either degrade different groups of azo dyes, nor to completely degrade the effluent (Jadhav *et al.* 2016). Since dye degradation is a complex process involving multiple steps and substrate specificity, the use of mixed bacterial populations shows higher degrees of biodegradation offering considerable advantage over the use of pure cultures (Dave *et al.* 2015; Jadhav *et al.* 2016).

It has been reported that the azo dyes-containing wastewaters are deficient in nutrient content; thus, their biodegradation, without any supplement of carbon or nitrogen sources, could be very difficult (Dave *et al.* 2015; Durruty *et al.* 2015). Glucose, starch, acetate, peptone and yeast extract (Bras *et al.* 2001; Chang *et al.* 2001; Chen *et al.* 2006; Dave *et al.* 2015) are among the most used nutrients, and they each show different levels of treatment efficacy. Dave *et al.* (2015) described different bacterial strains and their metabolic profiles with respect to different carbon and nitrogen sources. Each strain is capable of decolourizing azo dyes to differing degrees. This highlights the need to test different carbon sources for every microbial consortium.

The main objective of this work was to evaluate the biodegradation efficacy of a new bacterial consortium which had been collected from the dyeing factory sewer. Different carbon and nitrogen sources were added in order to enhance the degradation process of the wastewater. In this context, industrial starch, an economical by-product of potatoes industry, was studied as carbon source. Finally, the phytotoxicity of the degradation products was evaluated.

MATERIALS AND METHODS

Textile wastewater

The wastewater was collected from a local dyeing factory (Gama S. A., Mar del Plata, Argentina). On the sampling day, Direct Black 22 (DB22) was being used in the dyeing process (1 $g \cdot L^{-1}$), with the addition of sodium chloride (20 $g \cdot L^{-1}$) and sodium carbonate (2.14 $g \cdot L^{-1}$). Therefore, the wastewater contaminant mentioned in this paper refers only to DB22 (DB22, $C_{44}H_{32}N_{13}Na_3O_{11}S_5$; C. I. 35,435; CAS 6473-13-8; molecular weight of 1,083.97 $g \cdot mol^{-1}$), which is a tetra-azo dye.

Bacterial consortium

The bacterial consortium was collected from the manholes and inspection chamber of the sewer. The samples were taken with sterile cotton swabs and were immediately inoculated in nutrient agar plates and in LB broth supplemented with 200 $mg \cdot L^{-1}$ of DB22. Incubation was carried out during a 24 to 48 h period in the dark on a shaker plate (120 rpm). A second set of flasks were incubated without agitation. All cultures were maintained at 25 °C.

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Culture conditions

The bacterial consortium, which showed defined colour removal, was maintained in LB broth medium, without the addition of DB22, and was periodically renewed from the stock culture.

Glucose, starch, sodium acetate, yeast extract, peptone and sodium nitrate were independently tested as nutrient sources.

Degradation assays

Closed glass flasks (150 ml final volume) were filled with 110 ml of the wastewater, coming directly from the industrial facilities. Each flask was supplemented with 2 $g{\cdot}L^{-1}$ of glucose, starch, sodium acetate, yeast extract or peptone, respectively. All the treatments were then inoculated with 10 ml of stock culture. Samples were taken at regular time intervals to measure colour, DB22 and COD concentrations.

Treatments were as follows:

- Wastewater without nutrient sources (W)
- Wastewater + Glucose (W + G)
- Wastewater + Starch (W + S)
- Wastewater + Sodium Acetate (W + SA)
- Wastewater + Yeast Extract (W + YE)
- Wastewater + Peptone (W + P)

Wastewater without an inoculum was used as a control.

The W, W + G, W + S and W + SA treatments were also carried out with the addition of 1 $g{\cdot}L^{-1}$ of sodium nitrate.

Starch was provided by Mc Cain SA (Balcarce, Argentina). All other reagents used in the present work were commercial products of analytical grade from Sigma-Aldrich (St Louis, MO, USA).

Analytical methods

The colour was measured spectrophotometrically; for this, one millilitre of each flask was collected and clarified (centrifuged at 14,000 rpm for 10 min) in order to prevent absorbance interference from cellular or other suspended debris. Because the wastewater only contained the azo dye Direct Black 22 (see Textile wastewater section), the absorbance was measured at 480 nm since it is the wavelength with maximal absorbance in the visible region for this dye. The decolourization efficiency of the different treatments was expressed as described by Jasińska *et al.* (2012).

The concentration of the azo dye ($mg{\cdot}L^{-1}$) was estimated using a calibration curve, in agreement with Beer-Lambert's law, as reported by Durruty *et al.* (2015). The decolourization rate was expressed as mg of azo dye per volume and time ($mg{\cdot}L^{-1}{\cdot}h^{-1}$) (Chang & Kuo 2000; Chang *et al.* 2001; Chen *et al.* 2006; Hsueh & Chen 2008; Shah *et al.* 2016).

COD was measured using method 5520, Closed Reflux Method (APHA 1998). The pH was measured using a Hanna pH meter (HI 2,211 pH/ORP Meter) and conductivity was measured using a SPER SCIENTIFIC conductivity meter (Model 860032). Total phosphorus was determined using method 8190, commercial reagents (Hach Company, Loveland, CO, USA).

The growth of microorganisms was determined by measuring the dry weight. Once the decolourization runs were finished, the flasks containing the wastewater and the bacterial consortium were filtered onto a Whatman GF/A filter, rinsed twice with distilled water, and dried at 100 °C. Biomass was expressed as grams of dry weight per volume of reactor (L).

BOD was measured as previously described by the manometric respirometry test (OECD 1993) protocol. BOD Sensor System 6 (VELP Scientifica, Italy) was used to study the degradation of raw and treated wastewater. The activated sludge inoculum was obtained from an aerobic laboratory-scale (4.5 L) activated sludge reactor with partial biomass recycle (Ferro Orozco *et al.* 2010). To prevent nitrification, allylthiourea was added in all flasks at a concentration of 10 $mg{\cdot}L^{-1}$ (Stasinakis *et al.* 2008). Abiotic experiments were negative for all the tested compounds. A quantitative approximation to the biodegradability of a wastewater is given by the COD to biological oxygen demand at day five (BOD_5) ratio (COD/BOD_5). The criteria used consider that high, medium, and low COD to BOD_5 ratios are between 2.5 and 3.5, 2.0 and 2.5, and 1.5 and 2.0, respectively (Henze 2008). A lower (COD/BOD_5) ratio indicates a higher biodegradability.

All the reported values are the average of three measurements, and error bars represent the standard deviation.

Phytotoxicity studies

The phytotoxicity of the wastewater, before and after biological treatment, was evaluated measuring the percentage of seed germination and root elongation in the species *Lactuca sativa* (var. Gallega). This species is one of the most commonly used in literature because it is sensitive to the presence of phytotoxic compounds (Osma *et al.* 2010).

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First, LC50 of the effluent was calculated using the germination percentage. Serial dilutions of the effluent were done (50%, 25%, 16.7%, 12.5% and 6.25%). Five replicates, of 10 seeds each, were incubated in the presence of each dilution. Distilled water was used as control. Finally, the LC50 was calculated using the TRAP software version 1.30a (EPA 1999).

Once a dilution was selected, 10 seeds of *L. sativa* were placed in Petri dishes with a solution from each treatment. Controls were carried out with distilled water. All treatments were incubated in the dark at 25 °C until the length of the radicle was about 1.5 cm in the control seeds. The germination percentage was then calculated. Root elongation was measured using the Image J software (Image J 1.50i, Wayne Rasband, National Institute of Health, USA).

RESULTS AND DISCUSSION

Wastewater characterization and treatment alternatives

The textile wastewater used in this study was physicochemically characterized. It showed a conductivity of 24.2 ± 6 mS·cm^{-1} and a pH of 9.7 ± 0.1. In agreement with the literature (Shertate & Thorat 2014), the raw wastewater presented high levels of COD (6,510 mg L^{-1}) and BOD (1,075 mg L^{-1}). The high value of COD/BOD_5 ratio (6.1) indicated very low wastewater biodegradability. In addition, the wastewater presented a total phosphorous concentration of 8.7 ± 2.1 mg·L^{-1}.

To analyze the best conditions for the decolourization and degradation processes, the bacterial consortium was grown in LB broth supplemented with DB22 (200 mg·L^{-1}), with and without agitation. Decolourization was only observed in the last test sample, which is in agreement with other authors (Chang & Kuo 2000). For this reason, the following assays were performed without agitation conditions.

The decolourization of the textile wastewater by the bacterial consortium was studied using the wastewater alone (W) or supplemented separately with several nutrient sources: glucose (W + G), sodium acetate (W + SA), yeast extract (W + YE), peptone (W + P) or starch (W + S). The addition of sodium nitrate was evaluated as nitrogen source for the W, W + G, W + S and W + SA treatments. Table 1 shows the decolourization efficiency of the bacterial consortium, in the absence and in the presence of different carbon and nitrogen sources.

The addition of nitrogen, such as ammonium nitrate, ammonium chloride, peptone, urea, yeast extract and other sources, has been reported to be a beneficial factor that can promote the regeneration of nicotinamide adenine dinucleotide (NADH), which acts as an electron donor for the reduction of the azo bonds by the microorganisms (Chang & Kuo 2000; Henze 2008). However, in the present work, decolourization efficiency was not affected by the addition of a nitrogen source (Table 1). An analysis of variance (ANOVA) test was performed and no significant differences were observed in presence of sodium nitrate.

In the absence of an additional carbon source, the bacterial consortium was able to decolourize the raw wastewater with an efficiency of 77.63 ± 3.0% after 96 h. These results are similar to those reported by Balapure *et al.* (2016), who reported an 80% decolourization using anaerobic reactors and wastewater. Moreover, Amaral *et al.* (2014) obtained decolourization between 77% and 88% in an anaerobic reactor.

Table 1 | Decolourization kinetic rates and first order constant

	Decolourization percentage (%)		Initial decolourization rate (mg·L^{-1}·h^{-1})		First order k (h^{-1})	
Treatment	**−N**	**+N**	**−N**	**+N**	**−N**	**+N**
Glucose (W + G)	87.24 ± 2.5^{b}	84.59 ± 4.8^{e,b,f}	25.67 ± 3.62	22.63 ± 2.35	0.0182 ± 3.8E − 3	0.0104 ± 3.1E − 3
Sodium acetate (W + SA)	79.95 ± 2.1^{a}	81.89 ± 2.5^{a,f}	6.56 ± 0.57	6.45 ± 0.50	0.0169 ± 1.2E − 3	0.0188 ± 1.6E − 3
Yeast extract (W + YE)	74.40 ± 3.0^{c}	–	6.67 ± 1.11	–	0.0126 ± 6.024E − 4	–
Peptone (W + P)	68.22 ± 9.0^{d}	–	3.66 ± 0.53	–	0.0121 ± 6.808E − 4	–
Starch (W + S)	80.39 ± 3.1^{a}	79.21 ± 3.6^{a,c}	6.25 ± 0.56	5.96 ± 0.23	0.0161 ± 1.17E − 3	0.0156 ± 1.46E − 3
No carbon source (W)	77.63 ± 3.0^{a,c}	–	5.80 ± 0.31	–	0.0157 ± 9.446E − 4	–

+N means plus 1 g·L^{-1} of sodium nitrate and −N means without the addition of sodium nitrate.
$^{a–f}$ different letters means significant differences at $P < 0.05$ (Tukey's multiple comparisons test).

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The W + G treatment improved significantly the decolourization efficiency to 87.24 ± 2.55% at 96 h; Moreover, the addition of glucose also induced an increase in the decolourization rate, which was about 4 times higher (25.67 ± 3.62 mg of azo dye·L^{-1}·h^{-1}) than that obtained for the W treatment (5.8 ± 0.31 mg of azo dye L^{-1}·h^{-1}). Glucose metabolism produces reduced nucleotides (NADH, FADH) (Pathak *et al.* 2014) which could lead to an enhancement of the decolourization efficiency (Jain *et al.* 2012).

In the presence of starch (W + S treatment), the decolourization efficiency was about 80.39 ± 3.1%, indicating a slight improvement. However, it did not show a significant difference compared to the wastewater without an extra carbon source.

The presence of sodium acetate (W + SA, with and without $NaNO_3$), yeast extract (W + YE), and peptone (W + P) did not show an improvement on the decolourization process with respect to the W treatment. Several authors proposed that the simultaneous presence of the azo dye compound and an easily biodegradable carbon source could be detrimental to the decolourization due to the preferences of the microbial cells in assimilating the extra carbon source over the azo dye compound (Chang *et al.* 2001; Saratale *et al.* 2011).

Kinetic models which considered substrate saturation (like Michaelis-Menten) are widely used to fit biotransformations data (Blanch & Clark 1996; Wolski *et al.* 2012) and particularly to fit dye decolourization rates (Chang *et al.* 2001; Hsueh & Chen 2008; Durruty *et al.* 2015). Previous works, performed in a wide range of concentrations, showed that the decolourization rate fell, in the first order interval of Michaelis-Menten kinetics, below 300 mg of azo dye·L^{-1} (Chang & Kuo 2000; Chang *et al.* 2001). In this work, decolourization rates also fell, in this region, far below the saturation level (see Figure SM1, Supplementary Material, available with the online version of this paper). Thus, the data were fitted to a first order power law kinetics. The fitted first order kinetic constants are shown on Table 1. Figure 1 shows that up to 24 h of the assays, when the azo dye concentration was still high, a higher decolourization rate was observed. These results are in agreement with first order kinetics, as was postulated by Pearce *et al.* (2003). However, the first order power law does not seem to fit properly in all of the range. Figure 2 shows the logarithmic plot of DB22 concentration as a function of time for a) W and b) W + G, as examples. The slope decreased at the end of the run, indicating lower rates than those that would be predicted by a first order reaction. This phenomenon was previously observed by Wuhrmann *et al.* (1980), who attributed this effect to the toxicity of the metabolites produced during dye reduction. Table 2 summarizes the decolourization rates reported in literature for similar dye concentrations. Most of the decolourization rate values, obtained in this work, fell within the range of the reported data (Table 2). However, the decolourization on W + G treatment is clearly higher than those found in the literature (Table 2). Furthermore, since the observed decolourization rates are below the saturation rate (discussed above), a more accurate evaluation would be to compare the values of the first order kinetic constant versus the values of rmax/Ks reported. Thus, the fitted first order kinetic constant shown in Table 1 are in the upper range of the kinetic parameters previously reported (Chang *et al.* 2001; Hsueh & Chen 2008; Shah *et al.* 2016).

Considering the previous discussion: (a) the addition of glucose shows better decolourization kinetics, this would imply an additional cost in the industrial applications; (b) the bacterial consortium was able to decolourize the raw wastewater with an acceptable decolourization percentage, but there is insufficient information obtained regarding product toxicity; and (c) the use of starch does not imply an additional cost since it is a residue from the potato industry and is readily available. Here, analysis was focused on the W, W + G and W + S treatments.

The decolourization by the bacterial consortium caused a decrease of the pH from 9.7 ± 0.1 to 8.4 ± 0.3. 8.6 ± 0.4 and 8.6 ± 0.2 for W, W + S and W + G treatments, respectively (Table 3). A slight increase in the biomass concentration was also observed. Conductivity and total phosphorus concentrations did not show any observable changes (Table 3).

Generally, decolourization does not necessarily imply the completely azo dye degradation. For this reason, in order to analyze the decolourization vs. degradation behaviour, total degradation was measured as 'COD' as a function of time for W, W + S and W + G (Figure 3), and the results were compared with those described in Figure 1.

Figure 3 shows that the COD concentration decreased with time in each assay. For W + S, the COD concentration decreased in a similar way to decolourization (Figures 1 and 3), suggesting the simultaneous use of both substrates, starch and DB22. However, in the W + G treatment, the bacterial consortium appeared to decolourize DB22 first (Figure 1), then degrade the extra carbon source (Figure 3). Thus, the COD consumption for W + G treatment was slow and reached lower degradation levels than those observed in the W and W + S treatments. This result could be explained by the production of toxic metabolites during dye

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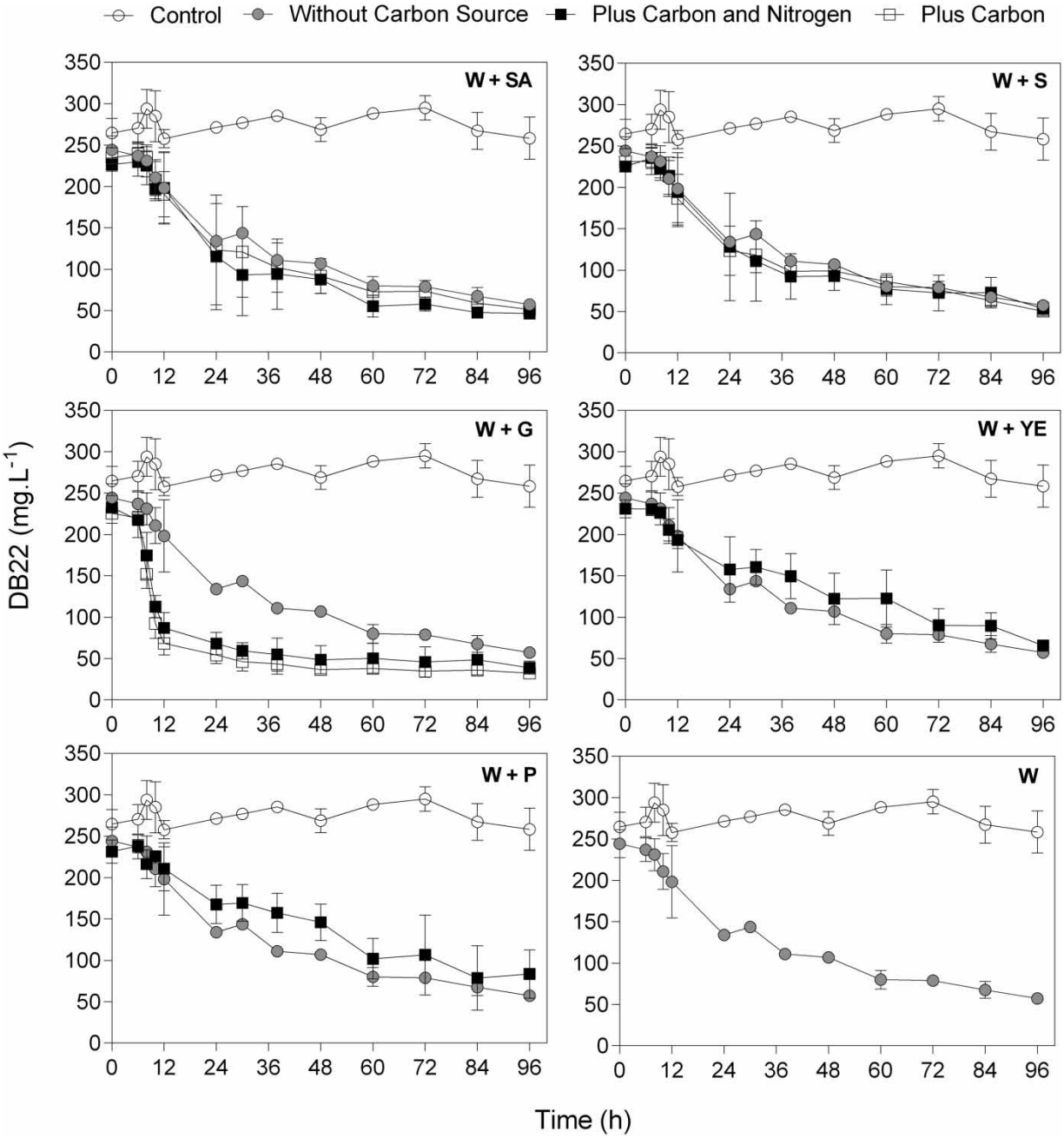

Figure 1 | Decolourization kinetic of the textile wastewater. The inoculum was 10% (v/v) of the microbial consortium (stock culture). Each point represents the mean value ± SD of three independent experiments. Control means wastewater without inoculation.

decolourization at early stages, which could inhibit the degradation of organic matter later (Wuhrmann *et al.* 1980). Finally, the behaviour of the bacterial consortium on the W treatment was similar to that observed in the W + S treatment; where decolourization and degradation processes occur simultaneously, but with a lower COD removal efficiency (Table 3).

Considering the initial COD degradation rate, the value obtained for W + S (Table 3) was within the range of the maximum rates reported in literature for anaerobic and microaerophilic bacteria. Isik & Sponza (2005) found a maximum COD degradation rate of 7.50 $gO_2 \cdot L^{-1} \cdot d^{-1}$ using glucose and starch as co-substrates for the degradation of a simulated textile wastewater. Shah *et al.* (2016) reported a

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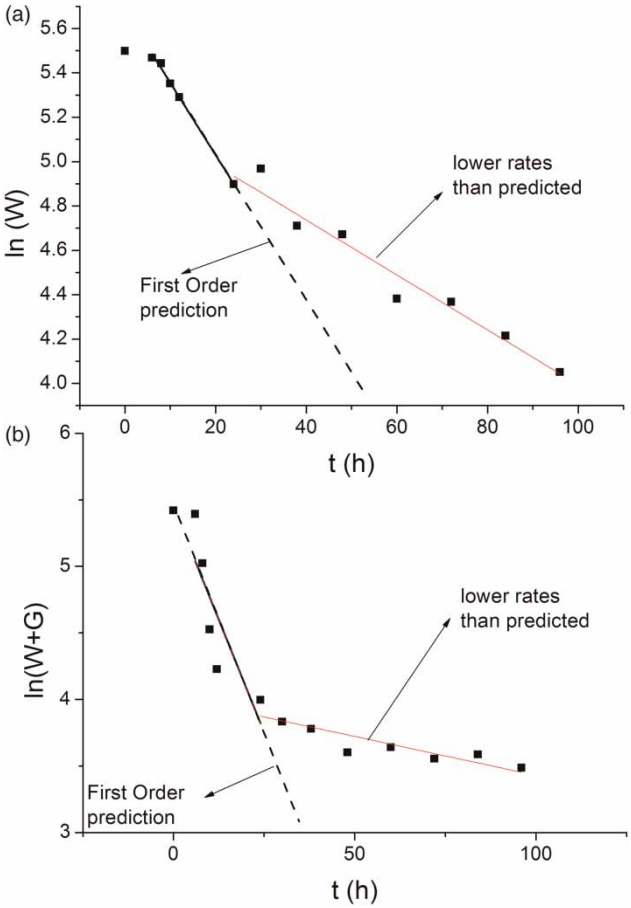

Figure 2 | Natural logarithm of Direct Black 22 concentration as a function of time for a) the wastewater inoculated with the bacterial consortium (W), and b) the wastewater inoculated with the bacterial consortium plus glucose (W + G).

value of 9.9 $gO_2 \cdot L^{-1} \cdot d^{-1}$ using glucose as co-substrate for the degradation of a simulated textile wastewater. However, in the present work, the initial value of the COD degradation rate for W + G was lower with respect to those described in the literature.

The different treatments were also evaluated by considering the evolution of the biodegradability of the assays. The biodegradability was followed by measuring the COD/BOD_5 ratio (Table 3). The addition of carbon sources, glucose and starch caused the increase of the initial values of COD and BOD_5. The low COD removal efficiency, during the W + G assay, and the high values of BOD_5, both give a low COD/BOD_5 ratio, even before (initial) and after (final) treatments, which indicate that the addition of glucose did not improve the degradation process.

As was described above, the behaviour of the decolourization and degradation performance by the bacterial consortium for W and W + S treatments, were similar. However, differences were observed in the biodegradability (Table 3). Although the addition of a carbon source caused an increase in the initial COD concentration, the final COD value for the W + S treatment was even lower than that obtained with the W treatment. The COD/BOD_5 ratio after treatment was also lower when starch was used as a carbon source, showing an improvement in the biodegradability of the wastewater (Figure 3, Table 3). The positive impact on the biodegradability observed after the W + S treatment indicates that this process can be used to improve the quality of the remained wastewater and later aerobic treatments can be applied. However, it is worth mentioning that the COD/BOD_5 ratio is an indication of the maximum possible biodegradability of the wastewater; thus, acclimation of the biomass or other operational factors during wastewater treatment works could affect the treatment efficiency.

Table 2 | Decolourization kinetics rates reported in the literature

Authors	Dye	Strain	Carbon source	Decolourization rate ($mg \cdot L^{-1} \cdot h^{-1}$)
Shah *et al.* (2016)	Mixture	Consortium VIE6	Glu (1 $g \cdot L^{-1}$), YE (1 $g \cdot L^{-1}$)	0.19
Chang *et al.* (2001)	Reactive Red 22	*P. luteola*	YE (3 $g \cdot L^{-1}$) + Glu (1.25 $g \cdot L^{-1}$) YE (2 $g \cdot L^{-1}$) Glu (1.25 $g \cdot L^{-1}$) YE (3 $g \cdot L^{-1}$) + Gly (1.25 $g \cdot L^{-1}$)	0.78 1.05 0.78 2.07
Hsueh & Chen (2008)	Methyl Orange, Methyl Red and Phenol	*P. luteola*	–	1.5–4.97
Chen *et al.* (2006)	Reactive Red 22	*P. luteola* and *E. coli*	YE (0.5%)	0–12
Chang & Kuo (2000)	Reactive Red 22	*E. coli*	–	17.5
This work	Direct Black 22	Consortium	Glu (2 $g \cdot L^{-1}$) –	25.67 ± 3.62 5.80 ± 0.31

YE: yeast extract; Glu: glucose; Gly: glycerol.

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Table 3 | Physicochemical parameters and biodegradability data

	Treatments		
Parameters	**W**	**W + S**	**W + G**
Final pH	8.4 ± 0.3	8.6 ± 0.4	8.6 ± 0.2
Final conductivity (mS·cm $^{-1}$)	24.7 ± 0.7	25.4 ± 0	25.2 ± 0.6
Final total phosphorus (mg·L^{-1})	9.0 ± 1.7	11.9 ± 1.3	6.9 ± 1.5
Biomass growth (mg·L^{-1})	7.0 ± 0.4	9.1 ± 3.4	8.5 ± 1.5
Initial COD (mg_{O2}·L^{-1})	6,510 ± 325.5	9,194 ± 459.7	7,624 ± 381.2
Final COD (mg_{O2}·L^{-1})	3,104 ± 155.2	2,525 ± 126.25	4,268 ± 213.4
COD removal efficiency (%)	52.32	72.52	44.02
Initial degradation rate (gO_2·L^{-1}·d^1)	2.709 ± 0.529	7.74384 ± 1.089	0.210 ± 8.66E − 03
First order degradation kinetic constant, k (h^{-1})	5.87E − 03 ± 1.48E − 03	1.233E − 02 ± 1.25E − 03	4.87E − 03 ± 1.28E − 03
Initial BOD_5 (mg_{O2}·L^{-1})	1,075 ± 0	2,570 ± 0	4,750 ± 0
Final BOD_5 (mg_{O2}·L^{-1})	740 ± 0	2,115 ± 56.6	2,665 ± 148.5
BOD removal efficiency %	31.2	17.71	43.89
Initial COD/BOD_5	6.1 ± 0	3.6 ± 0	1.6 ± 0
Final COD/BOD_5	4.2 ± 0	1.2 ± 0	1.67 ± 0.1

Initial and final means zero time and 96 h after treatment, respectively.

The addition of starch is a clear example of an additional carbon source enhancing the degradation of textile wastewater. This is in agreement with the established literature (Jain *et al.* 2012; Pathak *et al.* 2014). Taking into account that starch is frequently used as an additive in the textile finishing process (Dave *et al.* 2015), the use of microorganisms, which can use starch as a co-substrate, would be a beneficial option for the treatment of textile wastewater.

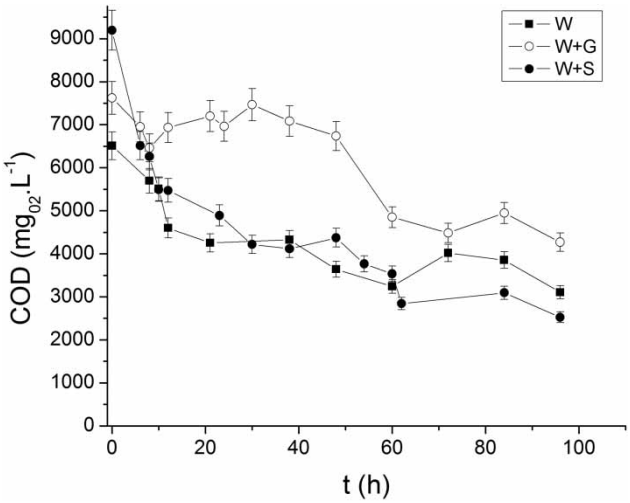

Figure 3 | COD degradation for: W, W + S and W + G treatments.

Phytotoxicity

As mentioned before, it is important to analyze the toxicity of azo dye product degradation. For this reason, the phytotoxicity of the raw (RW) and treated wastewater was assessed by observing the germination percentage and measuring the length of the radicle of lettuce (*Lactuca sativa*) seeds, which are sensitive to the presence of phytotoxic compounds. This is one of the most common phytotoxicity assays used in the literature (Osma *et al.* 2010). First, the EC50 of the raw wastewater for seed germination was evaluated (Figure 4). The results indicated that

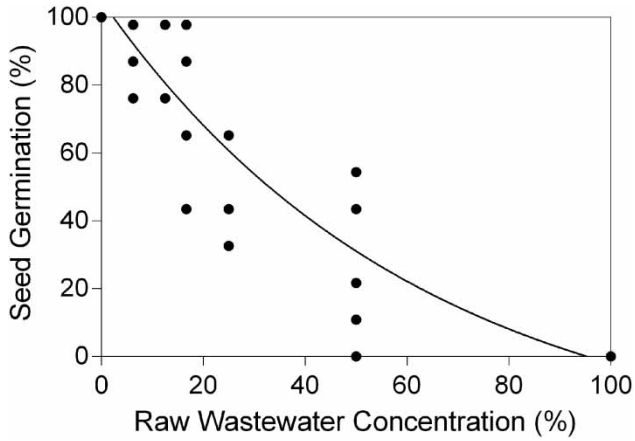

Figure 4 | The effect of textile wastewater on *Lactuca sativa* seed germination.

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Table 4 | Phytotoxicity analysis of raw wastewater and treated wastewater (W, W + G, and W + S)

Parameters	Control	RW	W	W + S	W + G
Germination (%)	96 ± 9.6 a	56.6 ± 17.5 b	71.1 ± 26.3 b	83.3 ± 19.8 a	66.7 ± 15.7 b
Radicle length (cm)	1.8 ± 0.5 a	0.4 ± 0.1 b	0.7 ± 0.2 c	0.7 ± 0.2 c	0.8 ± 0.2 c

*Values represent the mean ± SD of three independent experiments with five replicates each. Different letters means significant differences at $P < 0.05$ (Tukey's multiple comparisons test).

the concentration of wastewater for the phytotoxicity assay should be lower than 30%. Therefore, the following assays were carried out with a concentration of 25% of both raw and treated wastewater.

Table 4 shows that the raw wastewater was highly toxic with respect to the control (water), with respect to both the seed germination and the radicle length. The raw wastewater decreased the seed germination percentage by 40% and reduced the radicle length significantly (4.5 fold).

For seed germination, the biological treatments (W, W + S, W + G) caused an increase in the survival of the *Lactuca sativa* seeds, but significant differences were observed in the treatment with the addition of starch (W + S).

The radicle length was negatively affected by the raw wastewater, as mentioned before. Biological treatments (W, W + S and W + G) reduced the toxicity significantly by almost 50% with respect to the raw wastewater. However, none of these treatments reached the values observed in the control experiment. No significant differences were observed among the W, W + S and W + G treatments with respect to radicle length.

In the present work, the phytotoxicity and biodegradability results were in agreement. Considering biodegradability, the biological treatment with the addition of starch showed the best performance, and was also the only treatment that showed significant differences in seed germination rates.

CONCLUSIONS

The results presented in this work show that a bacterial consortium from a textile industry was able to decolourize and degrade the textile wastewater, without a pre-treatment or wastewater dilution. This consortium showed a higher resistance to the harsh conditions presented by the wastewater, such as elevated pH and salinity.

By considering the following:

(a) the consortium was able to decolourize and degrade the raw wastewater without an additional carbon source even though the products of the treatment presented low biodegradability;
(b) the use of glucose as additional carbon source increased the decolourization rate significantly, although it was still detrimental with respect to COD removal efficiency;
(c) the addition of starch improved the biodegradability;
(d) the phytotoxicity assay presented better results for the treatment in the presence of starch;
(e) the industrial starch is a low cost by-product of the local potato industries that is frequently used as an additive in some textile finishing processes.

It can be concluded that the addition of industrial starch to the biological process of azo dye degradation is an attractive alternative for the treatment of textile wastewaters.

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