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# Enhancement of textile wastewater decolourization and biodegradation by isolated bacterial and fungal strains

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**ABSTRACT**

This study was made to isolate bacterial and fungal strains from sludge samples obtained from a continuous stirred tank reactor treating indigo dye-containing textile wastewater. Twenty-two bacteria and four fungi were isolated and tested for the decolourization and the degradation of this effluent. The higher decolourizing and degrading strains were identified as *Bacillus cereus* (KEB-7) and *Bacillus pumilus* (KEB-10) for bacteria and *Aspergillus alliaceus* (KF-3) for fungi. Decolourization of 91%, 92%, and 93%, and COD removal of 90%, 93%, and 90% were achieved for the three strains, respectively. The three effective isolates were used in different combinations to examine the effect of their concerted metabolism on the decolourization and COD removal efficiencies. It was shown that the microbial consortium formed of the mixture of the three strains presented a significant improvement of textile wastewater decolourization (100%) and COD removal yields (98%) due to the synergetic reaction of bacterial and fugal strains.

***Keywords***: Textile wastewater; Decolourization; PCR-SSCP; Bacteria; Fungi

## 1. Introduction

Textile industries consume a considerable amount of water in their manufacturing processes. Considering both the volume and the effluent composition, the textile industry is rated as the most polluting among all industrial sectors. Important pollutants in textile effluents are mainly recalcitrant organics, dyes, toxicants and inhibitory compounds, surfactants, chlorinated compounds (AOX), pH and salts [1,2]. Alterations to their chemical structures can result in the formation of new xenobiotic compounds which may be more or less toxic than the potential compounds [3]. It has been proved that dyes have been identified as the most problematic compounds in textile effluents as they are difficult to remove due to their high water solubility and low exhaustion [4].

Numerous physical and chemical processes have been proposed for the treatment of coloured wastewater, e.g. precipitation, flocculation, and membrane filtration [5–7], although these treatment methods may result in the production of toxic by-products and/or require high levels of energy. Biological decolourization has been proposed as a less expensive and less environmentally intrusive alternative [3,8–10]. Many microorganisms belonging to different taxonomic groups of bacteria [11,12], actinomycetes [13] and algae [14] have been reported for their ability to decolourize these wastes. Although many reports are available in the literature

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regarding capability of pure cultures to decolourize textile wastewater containing dyes [15–18], but they do not find many applications in treatment systems for industrial effluent because of heterogeneity of the components in effluents depending upon production schedule. The treatment systems having mixed microbial populations are more effective due to concerted metabolic activities of microbial community [19,20]. Recently, bacterial strains which display good growth in aerobic or agitation culture have been described [21]. Mixed culture studies may be more appropriate for decolourization of dyes. About 80% of colour removal in effluent sample containing mixture of dyes was observed by using mixed bacterial culture [22]. An isolated microbial consortium removed 67–84% of colour from textile dye effluent after 44 h of cultivation [23].

This work aims to isolate bacterial and fungal strains which possessed the ability to decolourize indigo dye-containing textile wastewater. These isolates were used to develop a microbial consortium that can decolourize and biodegrade dyes at a faster time and can be used further to develop a continuous process for the treatment of textile processing industry wastewater containing a wide variety of textile dyes.

## 2. Material and methods

### 2.1. Origin of the microbial strains

The microbial source was obtained from an aerobic continuous stirred tank reactor (CSTR) treating textile wastewater which operated continuously without sludge recycling, at a constant temperature of 30°C. This reactor was inoculated with an acclimated microbial consortia obtained from a textile wastewater treatment plant. These inoculates were selected because of the large variety of microorganisms that could be found in the biomass degrading dyes in textile wastewater, and because mixed cultures offer considerable advantages. The reactor was feed with the indigo dye-containing textile wastewater (total COD: 1120 $mgL^{-1}$, colour: 1.4 unit $OD_{620\ nm}$ and pH 7.5), and operated with a variable wastewater loading rates ranged between 0.28 $gL^{-1}d^{-1}$ to 1.12 $gL^{-1}d^{-1}$. The reactor still maintained good performances in terms of colour and COD removal efficiencies above 90% and 85% respectively, suggesting that the microbial community present a high specific activity. Considering these results, some microorganisms possessing the ability to decolourize textile wastewater may be selected from this reactor.

### 2.2. Growth medium

The microbial source was cultivated in culture media either for bacteria (M1) or for fungi (M2). The medium M1 contained (in g/L): peptone 5, yeast extract 2.5, NaCl 5, (pH 7.0, 2% agar); and the medium M2 contained (in g/L): peptone 5, glucose 5 (pH 7.0, 1.5% agar), respectively [4].

### 2.3. Isolation and screening of textile wastewater decolourizing and biodegrading microorganisms

The two medium broths M1 and M2 were inoculated with 10% (v/v) of sludge sample in 250 ml flasks. The flasks were incubated at 30°C under shaking conditions (150 rpm). After 48 h of incubation, 1.0 ml of the culture broth was appropriately diluted two times and plated on agar plates of the same two mediums (M1-agar and M2-agar). The morphologically distinct bacterial and fungal isolates were selected for further studies. The pure culture stocks of these isolates were stored at 4°C. These isolates were screened for their ability to decolourize the textile wastewater diluted two times (total COD: 560 $mgL^{-1}$, colour: 0.7 unit $OD_{620\,nm}$ and pH 7.5). A loopful of growth form stock culture slope was inoculated into correspondent medium. After 24 h of incubation, the culture broth was transferred in 250 ml flasks containing 100 ml of the diluted effluent. The pH of the medium was adjusted to 7.0. After inoculation (10% v/v of the individual or combined isolates with an $OD_{600\,nm}$ of 0.8 at the start of the experiment), the flasks were incubated at 30°C under shaking conditions (150 rpm). The uninoculated control was also incubated to check the abiotic decolourization. The decolourization and COD removal efficiencies of these isolates were determined [24].

### 2.4. Decolourization and biodegradation assays

Carbon oxygen demand (COD) was measured following standard methods [25]. Colour was measured by an UV-vis spectrophotometer (Jenway UV visible spectrophotometer) at a wavelength of 620 nm in which maximum absorbance spectra were obtained in previous work for the same textile wastewater [1]. The pH was measured using a digital calibrated pH-meter (Hanna pH 210). All assays were performed in duplicate and compared with uninoculated controls [1]. Measured COD and colour absorbance values were used for calculation of COD removal and decolourization efficiencies. The decolourization was monitored by the percent (%) reduction on absorbance on comparison with control by means of the formula:

$$D = 100\ (D_i - D_t)/D_i$$

where $D$ is the decolourization of dye (in %), $D_i$ the initial concentration of dye, and $D_t$ the dye concentration along time. The COD removal was monitored by the percent reduction on COD on comparison with control by means

of the formula:

$$C = 100\ (C_i - C_t)/C_i$$

where $C$ is the COD removal of dye (in %), $C_i$ the initial COD and $C_t$ the COD along the time.

### *2.5. DNA analysis by PCR-SSCP and molecular identification of the isolates*

A suspension of each isolated colony was produced in 100 µl of sterile water. DNA was extracted from cells by the heat shock method [26]. The variable V3 region was amplified using a forward primer w49 (5′-ACGGTCCA GACTCCTACGGG) and a reverse primer w 34 (5′TTACC GCGGCTGCTGGCAC) [27]. SSCP analysis of each clone was performed using the ABI 310 Genetic Analyzer (Applied Biosystems), equipped with a capillary tube (47 cm × 50 µm) as described previously [27,28]. After assignment for each peak, several bacterial clones (effective ones) were sequenced as described previously [29]. The fungus isolates were identified by the Catholic University of Louvain in Belgium. The effective bacterial and fungal isolates were used in different combinations to determine the effect of their concerted metabolism on decolourization and COD removal efficiencies.

## 3. Results and discussion

### *3.1. Isolation and identification of textile wastewater decolourizing and biodegrading microorganisms*

The screening of microbial populations from the collected samples led to the isolation of 22 morphologically distinct bacteria and four fungal isolates responsible of the decolourization of the dye on M1 and M2 agar plates respectively. All bacterial isolates were analyzed for 16S rDNA with PCR-SSCP methods. Twenty-two SSCP patterns, each containing one dominant peak, were obtained and compared together to verify the similarity between them. In fact, seven different SSCP patterns were obtained, corresponding to seven different strains as shown in Fig. 1.

Six isolates (KEB-1, KEB-3, KEB-6, KEB-9, KEB-12 and KEB-17), four isolates (KEB-5, KEB-8, KEB-15 and KEB-21), two isolates (KEB-7, KEB-16), three isolates (KEB-11 , KEB-18 and KEB-20), two isolates (KEB-14 and KEB-19), three isolates (KEB-4, KEB-13, and KEB-22), and two isolates (KEB-2 and KEB-10) were attributed respectively to the SSCP patterns (1), (2), (3), (4), (5), (6) and (7).

All selected isolates were checked for their ability to decolourize and biodegrade indigo dye-containing textile wastewater. The results obtained are shown in Table 1. For bacterial isolates, decolourizations and COD removal

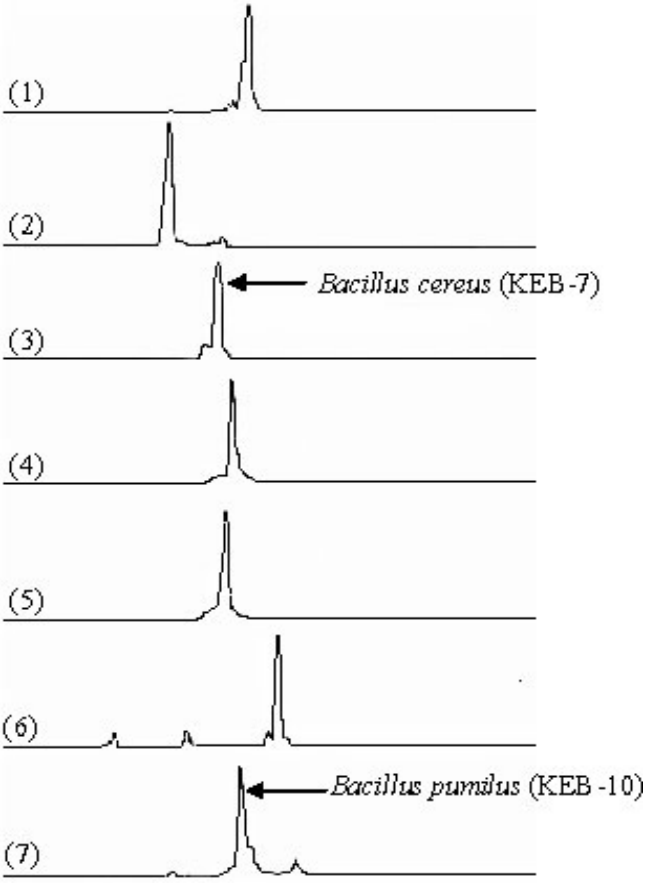


Fig. 1. Single strand conformation polymorphism patterns of bacterial 16S rDNA region amplification products of the bacterial isolates.

yields ranged between 45% (KEB 14) and 92% (KEB 10), and between 54% (KEB 14) and 93% (KEB 10), were respectively obtained after 72 h of incubation. For fungal isolates these yields ranged between 71% (KF-4) and 93% (KF-3), and between 64% (KF-2) and 90% (KF-3), respectively for the colour and COD removal efficiencies.

Three effective isolates were selected: two bacterial isolates designated KEB-7, KEB-10 and one fungal isolate designated KF-3. The isolate KEB-7 was identified as *Bacillus cereus*, the isolate KEB-10 was identified as *Bacillus pumilus* and the fungal strain KF-3 was identified as *Aspergillus alliaceus*. The decolourizations of 91%, 92%, and 93%, and a COD removal of 90%, 93%, and 90% were achieved for the three strains, respectively on 72 h of incubation. These removal efficiencies could be attributed to both degradation and absorption of the dye on surface cell [1]. To elucidate the decolourizations and COD removal efficiencies, the dye accumulation was checked by the extraction of dye from the cell pellets in methanol [4]. The bacterial cells in all of the above experiments remained white, indicating no textile wastewater was

Table 1
Textile wastewater decolourization and COD removal yields by bacterial and fungal isolates

| Isolates | Decolourization (%) after | | | | COD removal (% ) after | | | |
|---|---|---|---|---|---|---|---|---|
| | 12 h | 24 h | 48 h | 72 h | 12 h | 24 h | 48 h | 72 h |
| KEB-1 | 22 | 42 | 63 | 72 | 12 | 33 | 57 | 64 |
| KEB-2 | 26 | 45 | 69 | 88 | 34 | 59 | 76 | 89 |
| KEB-3 | 21 | 32 | 58 | 74 | 27 | 48 | 69 | 81 |
| KEB-4 | 18 | 34 | 53 | 66 | 15 | 29 | 44 | 60 |
| KEB-5 | 28 | 49 | 66 | 83 | 21 | 46 | 67 | 82 |
| KEB-6 | 22 | 41 | 58 | 71 | 27 | 48 | 64 | 78 |
| KEB-7 | 35 | 64 | 80 | 91 | 36 | 59 | 77 | 90 |
| KEB-8 | 29 | 52 | 69 | 82 | 26 | 46 | 68 | 86 |
| KEB-9 | 20 | 49 | 61 | 75 | 31 | 55 | 72 | 83 |
| KEB-10 | 30 | 57 | 83 | 92 | 30 | 51 | 81 | 93 |
| KEB-11 | 25 | 39 | 54 | 65 | 22 | 34 | 53 | 72 |
| KEB-12 | 32 | 49 | 56 | 72 | 31 | 47 | 61 | 76 |
| KEB-13 | 22 | 31 | 48 | 66 | 20 | 35 | 45 | 58 |
| KEB-14 | 13 | 24 | 34 | 45 | 18 | 30 | 41 | 54 |
| KEB-15 | 29 | 45 | 69 | 83 | 29 | 42 | 62 | 77 |
| KEB-16 | 30 | 48 | 67 | 89 | 33 | 54 | 61 | 85 |
| KEB-17 | 25 | 46 | 59 | 68 | 17 | 35 | 47 | 61 |
| KEB-18 | 28 | 40 | 52 | 64 | 24 | 43 | 56 | 73 |
| KEB-19 | 14 | 23 | 39 | 52 | 19 | 37 | 53 | 62 |
| KEB-20 | 19 | 43 | 59 | 73 | 29 | 47 | 64 | 79 |
| KEB-21 | 31 | 54 | 73 | 84 | 33 | 54 | 78 | 90 |
| KEB-22 | 18 | 37 | 53 | 65 | 22 | 41 | 57 | 71 |
| KF-1 | 32 | 50 | 66 | 82 | 26 | 46 | 68 | 80 |
| KF-2 | 22 | 42 | 63 | 72 | 12 | 33 | 57 | 64 |
| KF-3 | 28 | 57 | 83 | 93 | 23 | 59 | 83 | 90 |
| KF-4 | 26 | 43 | 55 | 71 | 27 | 48 | 54 | 65 |

Individual isolates have an $OD_{600\,nm}$ of 0.8 at the start of the experiment.

Table 2
Textile wastewater decolourization and COD removal yields by various combinations of the effective isolates

| Culture combinations | Decolourization (%) after | | | COD removal (% ) after | | |
|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h |
| KEB-7 + KEB-10 | 59 | 92 | 95 | 44 | 91 | 96 |
| KEB-7 + KF-3 | 78 | 94 | 97 | 54 | 89 | 96 |
| KEB-10 + KF-3 | 72 | 95 | 98 | 60 | 93 | 98 |
| CBF: KEB-7 +KEB-10 + KF-3 | 83 | 100 | 100 | 55 | 98 | 100 |

Individual isolates were mixed in equal proportion to achieve an $OD_{600\,nm}$ of 0.8 at the start of the experiment.

absorbed into the cell surface. But for all fungal experiments, the fungus cells turned blue in colour, indicating the absorption of the dye into the cell surface. In fact, the decolourization may be due to both biodegradation and absorption mechanisms. The same observations were mentioned earlier by Aksu et al. [30] and by Yesilada et al. [31].

### *3.2. Textile wastewater degradation and decolourization with different combinations of the effective microorganisms*

The three effective isolates were used in different combinations to examine the effect of their concerted metabolism on the decolourization and COD removal efficiencies, as shown in Table 2. For the individual isolates, colour and COD removal efficiencies are above

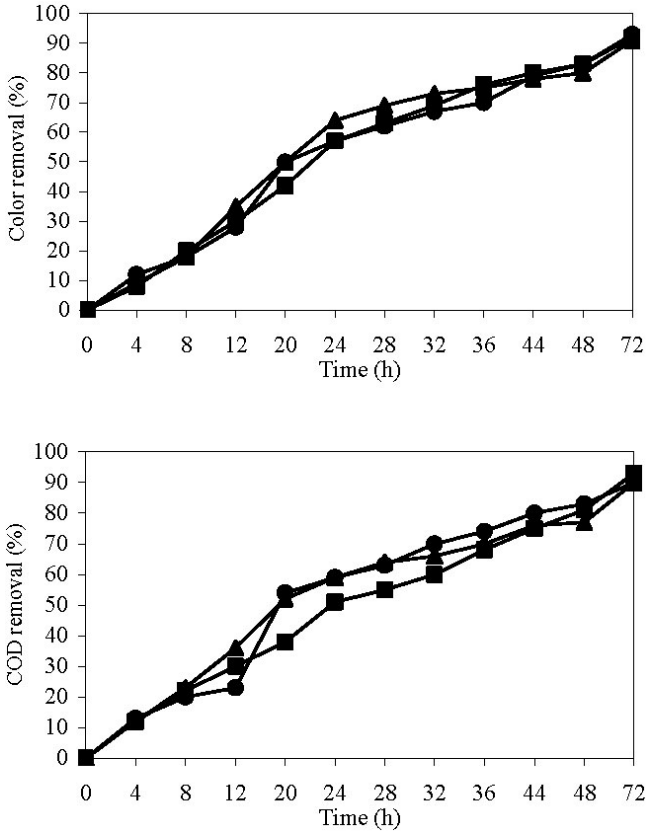


Fig. 2. Colour and COD removal of the single isolates KEB-7 (▲); KEB-10 (■); and KF-3 (●).

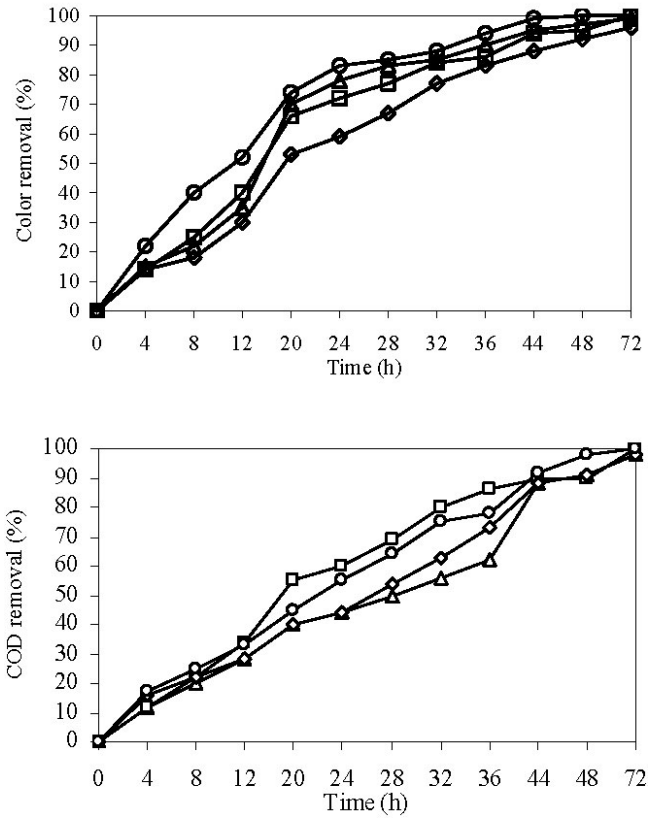


Fig. 3. Colour and COD removal of combined isolates KEB-7-KEB-10 (◊); KEB-7-KF-3 (Δ); KEB-10-KF-3 (□); CBF: KEB-7-KEB-10-KF-3 (○).

90% obtained after 72 h of incubation. The decolourization, COD removal yields and the reaction time were improved. In fact, when the isolates were combined these yields were enhanced and achieved in shorter time compared with the single strains. The highest yields were observed for the microbial consortium (CBF) with a total decolourization and a COD removal of 98% obtained only after 48 h of incubation (Figs. 2 and 3).

The decolourization and COD removal yields were remarkably increased, with the microbial consortium compared with individual and/or bacterial and fungal strains. However, the colour and COD removal of the CBF were 5% and 4% respectively higher than the combination of the two bacterial strains, and there were no significant differences between the two combinations of bacterial and fungal strains. The complete decolourization and COD removal yields were obtained in faster time than all other combinations, which might be attributed to the synergistic reaction between bacteria and fungus. This combination enhanced the decolourization and COD removal that is attributed both to bacterial enzymes and to extracellular enzyme–ligninolytic peroxidases typically produced by the fungi during secondary metabolism of the stationary phase and to the absorption of the dye into the cell surface [32–35]. In fact, fungal ligninolytic enzymes were stimulated by the enzymes of the bacterial strains. These enzymes caused the cleavage of the dye molecule to small organics that were easily degraded via oxidation, and which were consumed by microorganisms until near-complete COD removal [4].

These results are in agreement with other works which showed that individual strains could not completely degrade textile wastewater [36,37] such as *Streptomyces spp.* [38], *Phanerochaete chrysosporium* and *Pseudomonas luteola* [39] (Table 3). The CBF may be effective for the treatment of textile wastewaters considering the results of a total colour removal and a 98% COD removal obtained only on 48 h of incubation compared with an yields of 80% of colour removal obtained by the microbial bacterial cultures used by Nigam et al. [22], treating mixture of azo and diazo reactive dyes, and the yields obtained by Banat et al. [23], treating textile dyes by an isolated bacterial cultures with a colour removal ranged between 67% and 84% (Table 3). This microbial consortium treating the

Table 3
Decolourization characteristics of textile wastewater containing dyes

| Strain | Dye | Concentration (mgL/1) | Colour removal (%) | Cultivation time | Reference |
|---|---|---|---|---|---|
| *Phanerochaete chrysosporium* | Azo | 150 mgL/1 | 27–99 | 15 d | [38] |
| *Streptomyces spp.* | Azo | 50 mgL/1 | 0–90 | 15 d | [38] |
| *Pseudomonas luteola* | Red G | 150 | 37.4 | 4 d | [39] |
| | RBB | | 93.2 | 4 d | |
| | RP2B | | 92.4 | 4 d | |
| | V2RP | | 88 | 4 d | |
| Mixed bacterial cultures | Mixture of azo- and diazo-reactive dyes | 0.5 mM | 80 | 4 d | [22] |
| Isolated bacterial cultures | Textile dyes | 0.5 mM | 67–84 | 44 h | [23] |
| Microbial consortium | 4BS | 50 mgl/1 | 99.1 | 24 h | [4] |
| Immobilized microbial consortium | 4BS | 50 mgl/1 | 99.6 | 6 h | [4] |
| *Bacillus pumilus* (KEB7) | Textile wastewater containing indigo dye | 560 mgL/1[a] | 91 | 3d | This work |
| *Bacillus cereus (*KEB10) | Textile wastewater containing indigo dye | 560 mgL/1[a] | 92 | 3d | This work |
| *Aspergillus alliaceus (*KF3) | Textile wastewater containing indigo dye | 560 mgL/1[a] | 93 | 3d | This work |
| Microbial consortium (CBF) | Textile wastewater containing indigo dye | 560 mgL/1[a] | 100 | 2d | This work |

[a]Total COD.

indigo dye-containing textile wastewater with an initial concentration of 560 mg COD/L, presents the same colour removal efficiencies with the microbial consortium used bye He et al. [4], treating the azo dye Direct Fast Scarlet 4BS, and using an initial concentration of 50 mg/L. Compared with other works, this CBF may be considered effective for the treatment of highly concentrated textile wastewater and in shorter times [4].

## 4. Conclusions

The screening of the microbial populations from the collected sludge reactor samples led to the isolation of twenty two morphologically distinct bacterial and four fungal isolates responsible of the decolourization of the dye. The higher decolourizing and degrading textile wastewater strains were identified as *Bacillus cereus* and *Bacillus pumilus* for bacteria and *Aspergillus alliaceus* for fungi. The decolourization of 91%, 92%, and 93%, and a COD removal of 90%, 93%, and 90% were achieved for the three strains, respectively. The decolourization, COD removal yields and the reaction time were remarkably improved with the microbial consortium compared with individual and/or bacterial and fungal strains, due to the synergetic reaction of bacterial and fugal strains leading to the complete mineralization of the textile wastewater.

The high colour (100%) and COD (98%) removal efficiencies of this microbial consortium indicated its potential use in antipollution treatments. However, only a better understanding of the mechanisms used will allow applying it to the cleaning up aquatic terrestrial environments.

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