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Effect of nitrogen and carbon sources on Indigo and Congo red decolourization by *Aspergillus alliaceus* strain 121C

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ABSTRACT

The decolourizing ability of *Aspergillus alliaceus* 121C was investigated on solid medium. The effects of nitrogen (N), carbon (C) sources and supplements on the decolourization of Indigo and Congo red dyes were studied. It has been shown that both the nature and the quantity of available N- and C-sources exert an influence on growth and decolourization. For the six N-sources (NH_4Cl, Diammonium Tartrate, urea, malt extract, peptone and yeast extract) tested for Congo red decolourization, 8 mM yeast extract provided the higher decolourized zone diameter (80 mm) and colony diameter (80 mm). 12 mM urea provided the higher decolourized zone diameter (76 ± 2 mm) and colony diameter (80 mm) for Indigo decolourization. For the C-sources tested (glucose, starch, glycerol and lactose), above 12.5 mM of glucose and 62.5 mM of starch provided the higher decolourized zones diameters of 80 mm and 77 ± 3 mm for Indigo and Congo red, respectively. When the fungi was grown in liquid medium containing optimum carbon and nitrogen sources supplemented with oak sawdust and wheat bran, more than 98.6% and 98% of colour removal are obtained for Indigo and Congo red dyes, respectively. The detection of ligninolytic enzymes proved that laccase and lignine-peroxidase (LiP) are the two enzymes responsible of the decolourization of the two dyes.

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1. Introduction

Various dyes are used in textile, food, papermaking and cosmetic industries. Textile mills discharge large volumes of effluents that are coloured due to the presence of dyes in particular [1]. In addition to their visual effect and their adverse impact in terms of chemical oxygen demand, many dyes are toxic, mutagenic and carcinogenic [2–4]. Dyes are identified as the most problematic compounds in textile effluents due to their high water solubility and low degradability [5–7]. Moreover, the frequently high volumetric rate of industrial effluent discharge in combination with increasingly stringent legislation, make the search for appropriate treatment technologies an important priority [8,9].

Numerous processes have been proposed for the treatment of coloured wastewater, e.g. precipitation, flocculation, coagulation, adsorption and wet oxidation [10,11]. All these methods have different colour removal capabilities, capital costs and operating speed. Among these methods coagulation and adsorption are the commonly used; however they create huge amounts of sludge which become a pollutant on its own creating disposal problems [12]. So, the need of efficient and economic processes to treat these effluents increases. As a consequence, there has been a growing interest in biotechnological processes. It has been proved that white rot fungi (WRF) produce non-specific lignin-degrading enzymes which degrade a wide range of organic pollutants including textile dyes [2,5,13,14]. Recently, potential applications of WRF are gaining increasing importance on the decolourization of various dyes such as *Phlebia tremellosa*, *Irpex lacteus*, *Pleurotus ostreaus*, *Trametes modesta* [15], *Pleurotus sajor-caju*, *Pycnoporus sanguineus*, *Phanerochaete chrysosporium* [5], *Trametes versicolor* [16,17], *Phlebia brevispora*, *Lentinula edodes*, *Trametes villosa*, *Pleurotus ostreatus* and *Ceriporiopsis subvermispora* [18], which were widely used and they proved that they can play an important role in the dye decolourization [4,19].

This decolourizing ability is due to that white rot fungus through catalytic action of extracellular ligninolytic enzymes that are induced under secondary metabolic conditions. Several fungi have the capability to decolourize industrial effluents containing dyes via adsorption to biomass without degradation or through complete aerobic degradation [20]. The efficiency of the decolourization process can be improved by carefully selecting the operational conditions such as the addition of suitable co-substrates in the culture

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medium especially carbon, nitrogen sources and microelements [15,21].

Dye decolourization by fungi during growth has been widely employed to identify their ligninolytic enzymatic activity [18,22,23]. No earlier publications concerning decolourization of dyes using *Aspergillus alliaceus* has been cited in the literature. However, in this study, the decolourizing ability of this fungus using Indigo and Congo red dyes will be investigated. These dyes are extensively used in local textile dyeing plants and they are water soluble and their concentration easily can be measured spectrophotometrically. The effect of substrate nutrient concentration and supplement on fungus decolourizing activity was studied, and the produced enzymes were investigated on the optimized liquid medium.

2. Materials and methods

2.1. Reagents

All chemicals and reagents used were of analytical reagent grade. 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonate (ABTS), H_2O_2, Indigo, Congo red, sodium malonate, sodium succinate, veratryl alcohol and sodium tartarate were purchased from Sigma (Chemie, Germany). The malt extract, peptone and yeast extract were purchased from Scharlau (Chemie, S.A. Barcelona). The peptone, malt extract and yeast extract contained 11%, 0.8% and 11% N, respectively. NH_4Cl, Diammonium Tartrate, urea, $MnSO_4$, H_2O, were purchased from Aldrich (Steinheim, Germany). Glucose, starch, glycerol and lactose were purchased from Fluka (Neu-Ulm, Germany). Oak sawdust, wheat bran and wheat straw were obtained from the local market.

2.2. Fungal strain

The fungus was isolated from an aerobic sludge collected from a plant treating textile wastewater in Tunisia. Strain 121C is identified according to THOM AND CHURCH as *A. alliaceus* (molecular identification by catholic university of Louvain Belgium). The culture was maintained on Sabouraud dextrose agar (typical formula in g/l: glucose, 40; mycological peptone, 10; agar, 15) at 4 °C.

2.3. Culture conditions

The optimisation of N, C-sources and supplements levels were performed on agar plates (80 mm in diameter, 25 ml medium/Petri dish) containing different N- and C-sources, agar (20 g/l) [23,24], Indigo or Congo red dyes and the other nutrients. These two dyes used were moderately toxic. As a consequence, experiments were performed at lower dye concentration of 0.15 g/l [14,25].

The N- and C-concentrations dependence of Indigo and Congo red decolourizations were investigated on media containing Mn (20 μM) in the form of $MnSO_4$, H_2O. Six different N-sources were tested. Three inorganic N-sources (NH_4Cl, Diammonium Tartrate and urea), and three organic N-sources (malt extract, peptone and yeast extract), in the concentration range of 0–20 mM. Then four different C-sources were tested (glucose, starch, glycerol and lactose) in concentration range of 0–62.5 mM. The effects of supplementation on decolourization and growth using oak sawdust, wheat bran and wheat straw were studied with different combinations (Table 1), on basis of the results obtained with the N- and C-sources in the aim to induce ligninolytic enzymes. These supplements were added in gunpowder to the medium with a concentration of 2% (w/v) [24]. The pH of the medium was adjusted to 5.4 (optimum pH for fungus growth) before sterilisation. All media were sterilised by autoclaving at 120 °C for 20 min, except those containing oak sawdust, wheat bran and wheat straw, which were sterilised for 50 min. Agar disks of the same size (8 mm diameter) were cut with a sterile metal tube, from the edges of the growing colonies on the agar plates covered by the mycelium. The experimental agar plates containing the appropriate nutrients were run in triplicate and they were inoculated with these agar disks, and then incubated at 30 °C (optimum temperature for fungus growth). The diameters of colonies and the decolourized zones are measured in mm for each N, C-source and supplement [24,26].

Table 1
The different combinations tested of the used supplements

Experiments	Wheat bran	Oak sawdust	Wheat straw
A	–	–	–
B	+	–	–
C	–	+	–
D	–	–	+
E	+	+	–
F	+	–	+
G	–	+	+
H	+	+	+

(+) or (–) signs denote whether the supplements was present in the medium or not.

2.4. Enzymes assays

2.4.1. Cultivation of strain

The extracellular ligninolytic enzymes produced by this fungus were investigated in liquid medium containing, 20 μM Mn in the form of $MnSO_4$, H_2O and 150 mg/l of the dye with the appropriate concentrations of nitrogen, carbon source and supplements. The pH of the autoclaved medium was adjusted to 5.4. Five plugs of fungal mycelium were inoculated into 300 ml of the appropriate medium in 500 ml conical flasks which were performed in triplicate and placed on a rotary shaker at 200 rpm at 30 °C. A control culture, without fungi, was also established.

2.4.2. Enzyme extraction and decolourization assays

The culture broth was first filtered to remove the fungal pellets and the remaining supplement, and then centrifuged at 10,000 rpm for 15 min. The resulting clear solution was used for enzyme activities assays and dye decolourization experiments [12].

The concentration of Indigo and Congo red were measured at wavelength corresponding to the maximum absorbance λ_{max} (650 nm for Indigo [19] and 500 nm for Congo red [27]), by means of UV–vis spectrophotometer (Jenway UV visible spectrophotometer). The decolourization was determined by the percent (%) of absorbance reduction as presented in the following equation:

$$D = \frac{100(C_i - C_t)}{C_i}$$

where D is the decolourization of dye (in %), C_i is the initial concentration of the dye and C_t is the dye concentration along the time.

2.4.3. Enzymatic activity analysis

Supernatants from the final optimum decolourization medium were analyzed for lignin-peroxidase (LiP), manganese-peroxidase (MnP) and laccase activities. In all cases, the reaction mixture contained 100 μl crude enzyme extract in a final volume of 2 ml.

Laccase activity was determined by monitoring the oxidation of 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) at 420 nm following the method of Shin and Lee [28]. An aliquot of

Table 2
Growth and dye decolourization on media containing Indigo dye and six N-sources used in different concentrations

Nitrogen source (mM)	Indigo (150 mg/l)													
	Decolourized zone diameter after 9 days of culture (mm)						*p*-Value	Colony diameter after 9 days of culture (mm)						*p*-Value
	NH_4Cl	DT	Urea	ME	PE	YE		NH_4Cl	DT	Urea	ME	PE	YE	
0	50 ± 2	50 ± 1	50 ± 2	50 ± 3	50 ± 2	50 ± 2	1	57 ± 3	56 ± 4	58 ± 2	58 ± 2	56 ± 1	57 ± 2	0.8537
4	65 ± 1	40 ± 2	66 ± 1	65 ± 1	60 ± 3	72 ± 3	0	74 ± 1	46 ± 2	73 ± 3	72 ± 2	67 ± 2	80	0
8	66 ± 1	41 ± 2	70 ± 2	66 ± 2	64 ± 1	70 ± 2	0	76 ± 3	49 ± 1	77 ± 2	74 ± 1	74 ± 3	80	0
12	70 ± 2	39 ± 1	76 ± 2	69 ± 1	58 ± 2	65 ± 3	0	78 ± 2	45 ± 2	80	77 ± 2	63 ± 1	74 ± 3	0
16	71 ± 1	39 ± 1	72 ± 1	73 ± 2	57 ± 2	64 ± 2	0	80	44 ± 1	80	80	66 ± 2	74 ± 2	0
20	69 ± 1	43 ± 2	72 ± 2	74 ± 1	55 ± 4	60 ± 1	0	80	48 ± 2	80	80	67 ± 1	68 ± 1	0
p-Value	0	0.00004	0	0	0.00046	0		0	0.00033	0	0	0	0	

PE: peptone; DT: Diammonium Tartrate; ME: malt extract; YE: yeast extract. The diameters of the colonies and the decolourized zones are reported as the average of the results on three samples. *p* values were determined for the decolourized zones and colonies diameters for each source and between different sources.

the enzyme solution (100 μl) was incubated in 1 ml of 100 mM sodium succinate buffer (pH 4.5) containing 2 mM ABTS at 30 °C. The oxidation was followed via the increase in absorbance at 420 nm (ε_{420} = 36,000 M^{-1} cm^{-1}). The blanks received the buffer in place of ABTS. One unit of enzyme activity was defined as the amount of enzyme required to produce an absorbance increase of one unit per minute per milliliter of the reaction mixture.

MnP activity was measured by monitoring the oxidation of 1 mM $MnSO_4$ in 50 mM sodium malonate buffer (pH 4.5) in the presence of 0.1 mM H_2O_2 [29], via the increase in absorbance at 468 nm (ε_{468} = 49,600 M^{-1} cm^{-1}). One unit of MnP activity was defined as the amount of enzyme required to produce an absorbance increase of one per minute per milliliter of the reaction mixture. The blanks contained all reagents except $MnSO_4$.

LiP activity was determined by the method of Tien and Kirk [30], by measuring the absorbance increase at 310 nm (ε_{310} = 9300 M^{-1} cm^{-1}). The reaction mixture (2 ml) containing 4 mM veratryl alcohol in 10 mM sodium tartarate buffer (pH 3) incubated with 100 μl of the culture fluid at 30 °C. The reaction was initiated with addition of suitable amount of 0.2 mM H_2O_2. The blanks contained buffer in place of varatryl alcohol. One unit of LiP activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of veratraldehyde per minute under the assay conditions. Data presented are the average of triplicate measurements.

2.5. Statistical analysis

To evaluate the influence of the nature, the concentration of each N- or C-sources, and the influence of the supplement upon the decolourization and the growth, the ANOVA analysis were performed, and the means of the significantly different main effects were compared at $p < 0.05$ [15,31].

3. Results

3.1. Effect of nitrogen source on the decolourization of the Indigo and Congo red dyes

The solid medium used does not generally contain sufficient N- and C-sources. For adequate aerobic decolourization process an experiment was therefore conduct to screen the most essential nutrients for decolourization of Indigo and Congo red. The decolourized zones diameters, colonies diameters on the media containing these dyes and the different N-sources are listed in Tables 2 and 3. For the six N-sources tested for the decolourization of the Indigo, NH_4Cl, malt extract and urea provided the highest growth and decolourization, especially at concentrations above 16 mM, 16 mM and 12 mM, respectively (Table 2) and they ensured the fastest decolourizations. Peptone and yeast extract at high nutrient N-concentrations inhibited the decolourization shown by the decrease of the decolourized zones diameters with the increase of the N-concentrations. But for urea, malt extract and NH_4CL, the decolourization is stimulated by high nitrogen concentrations. The Diammonium Tartrate inhibited the growth and the decolourization in all concentrations compared with the sample without nitrogen source.

The highest decolourized zones diameters of Congo red were obtained with 8 mM yeast extract (80 mm), 16 mM malt extract (78 ± 1 mm), 4 mM Diammonium Tartrate (74 ± 1 mm) and 4 mM peptone (76 ± 2 mm) and its occurred only on 9 days. When urea was used as nitrogen source, limited decolourization of Congo red was observed.

The ANOVA analysis of the data indicated that the decolourized zones and colonies diameters were statistically significant ($p < 0.05$) for all tested sources (Tables 2 and 3). This significance is observed between all tested sources for the same dye, and between all concentrations tested for different N-sources.

Table 3
Growth and dye decolourization on media containing Congo red dye and six N-sources used in different concentrations

Nitrogen source (mM)	Congo red (150 mg/l)													
	Decolourized zone diameter after 9 days of culture (mm)						*p*-Value	Colony diameter after 9 days of culture (mm)						*p*-Value
	NH_4Cl	DT	Urea	ME	PE	YE		NH_4Cl	DT	Urea	ME	PE	YE	
0	56 ± 3	56 ± 4	56 ± 2	56 ± 2	56 ± 1	56 ± 2	1	65 ± 3	65 ± 3	65 ± 3	65 ± 3	65 ± 3	65 ± 3	1
4	63 ± 2	74 ± 1	52 ± 2	74 ± 2	76 ± 2	78 ± 2	0	72 ± 2	80	60 ± 3	74 ± 2	80	80	0
8	66 ± 2	66 ± 2	43 ± 1	73 ± 3	75 ± 2	80	0	74 ± 2	73 ± 2	55 ± 1	73 ± 3	80	80	0
12	69 ± 1	60 ± 3	34 ± 3	72 ± 2	74 ± 1	80	0	78 ± 1	70 ± 1	50 ± 1	72 ± 2	80	80	0
16	73 ± 1	48 ± 3	32 ± 2	78 ± 1	72 ± 3	80	0	80	60 ± 1	48 ± 2	78 ± 1	80	80	0
20	77 ± 1	53 ± 2	35 ± 2	73 ± 2	69 ± 1	80	0	80	61 ± 2	48 ± 1	73 ± 1	80	80	0
p-Value	0	0	0	0	0	0		0	0	0	0.00049	0	0	

PE: peptone; DT: Diammonium Tartrate; ME: malt extract; YE: yeast extract. The diameters of the colonies and the decolourized zones are reported as the average of the results on three samples. *p* values were determined for the decolourized zones and colonies diameters, for each source and between different sources.

Table 4
Growth and dye decolourization on media containing Indigo dye and four C-sources used in different concentrations

Carbon source (mM)	Indigo (150 mg/l)									
	Decolourized zone diameter after 9 days of culture (mm)				*p*-Value	Colony diameter after 9 days of culture (mm)				*p*-Value
	Glucose	Starch	Glucerol	Lactose		Glucose	Starch	Glucerol	Lactose	
0	54 ± 1	54 ± 3	54 ± 1	54 ± 2	1	66 ± 2	66 ± 3	66 ± 4	66 ± 2	1
12.5	75 ± 2	63 ± 2	53 ± 3	56 ± 3	0.00003	80	71 ± 4	64 ± 3	66 ± 3	0.00065
25	80	66 ± 1	58 ± 2	53 ± 2	0	80	75 ± 3	71 ± 1	68 ± 2	0.0027
37.5	80	70 ± 3	59 ± 1	58 ± 2	0	80	78 ± 2	73 ± 2	64 ± 2	0.00001
50	80	71 ± 2	64 ± 2	57 ± 3	0	80	80	74 ± 2	69 ± 2	0.00003
62.5	80	74 ± 2	68 ± 3	61 ± 3	0.00005	80	80	80	73 ± 2	0.00005
p-Value	0	0	0.00001	0.263		0	0.0009	0.0004	0.00483	

The diameters of the colonies and the decolourized zones are reported as the average of the results on three samples. *p* values were determined for the decolourized zones and colonies diameters, for each source and between different sources.

3.2. Effect of carbon source on the decolourization of the Indigo and Congo red dyes

The effects of carbon sources were investigated on media containing 20 μM Mn in the form of $MnSO_4$, agar (20 g/l) and two optimum N-sources previously obtained; urea (12 mM) and yeast extract (8 mM), for the decolourization of Indigo and Congo red, respectively. Four different C-sources were tested (glucose, starch, glycerol and lactose) in concentrations range of 0–62.5 mM. The diameters of the decolourized zones and the colonies diameters with the different C-sources are listed in Tables 4 and 5. When *A. alliaceus* was incubated with Indigo and without carbon sources, growth was limited compared with the media supplemented with the carbon sources. In presence of carbon sources an improvement of decolourization was observed. Glucose and starch provided the highest decolourized zones diameters, especially above 25 mM (80 mm) and 62.5 mM (74 ± 2 mm), respectively, and ensured the fastest complete decolourization that is occurred between 9 and 10 days. For the glycerol and lactose the decolourized zones diameters increased with the increase of the C-concentrations. For the four C-sources tested for the decolourization of the Congo red, the results obtained are similar to those obtained with the Indigo. Lactose did not display a concentration-dependant effect on the decolourization on the range of 12.5–62.5 mM of C-concentrations. In fact, the ANOVA analysis showed that no significance ($p > 0.05$) observed between all lactose concentrations on the decolourized zone diameter of the two dyes. The obtained *p* values were 0.263 and 0.07585 for Indigo and Congo red, respectively. These analyses showed also that decolourized zones and colonies diameters were statistically significant ($p < 0.05$) between all tested sources for the same dye and between all concentrations tested for different C-sources except those of lactose source (Tables 4 and 5).

3.3. Effect of the addition of the supplement on the decolourization of the Indigo and Congo red dyes

The optimal N- and C-sources previously set up were used (12 mM urea, 25 mM glucose for Indigo and 8 mM yeast extract, 25 mM glucose for Congo red). The obtained results are presented in Fig. 1. It was shown that the addition of natural supplements exhibit a positive effect on Indigo and Congo red decolourizations. In fact, the highest and fastest decolourized zones diameters were observed in the presence of the three supplements (80 mm for the two dyes below 7 days).

Oak sawdust and wheat bran enhanced the decolourization of the two dyes, but wheat straw did not affect the decolourization. In fact, when it is supplemented to the medium, the decolourized zones diameters obtained with and without this supplement are similar.

The ANOVA analysis of the data indicated that the decolourized zones and colonies diameters for the same dye were statistically significant ($p < 0.05$) for all tested combinations of the three supplements. No significance is observed between all tested combinations for the two dyes, except the combinations B and E for decolourized zones diameters and the combination F for colonies diameters in which the data were statistically significant ($p < 0.05$).

3.4. Detection of enzymes responsible of decolourization on liquid medium

The detection of enzymes responsible for the decolourization was investigated in liquid medium containing optimum N- and C-sources as described previously (12 mM urea, 25 mM glucose for Indigo and 8 mM yeast extract, 25 mM glucose for Congo red), and supplemented with oak sawdust and wheat bran. The fungal

Table 5
Growth and dye decolourization on media containing Congo red dye and four C-sources used in different concentrations

Carbon source (mM)	Congo red (150 mg/l)									
	Decolourized zone diameter after 9 days of culture (mm)				*p*-Value	Colony diameter after 9 days of culture (mm)				*p*-Value
	Glucose	Starch	Glucerol	Lactose		Glucose	Starch	Glucerol	Lactose	
0	61 ± 1	61 ± 4	61 ± 1	61 ± 3	1	68 ± 1	68 ± 2	68 ± 4	68 ± 1	1
12.5	71 ± 1	60 ± 2	60 ± 2	63 ± 1	0.0045	80	73 ± 2	73 ± 2	70 ± 1	0.0024
25	73 ± 2	65 ± 2	59 ± 3	64 ± 2	0.0052	80	80	72 ± 4	72 ± 2	0.0202
37.5	74 ± 1	68 ± 1	65 ± 1	62 ± 1	0	80	80	80	75 ± 1	0
50	77 ± 1	74 ± 1	68 ± 1	66 ± 2	0.00002	80	80	80	73 ± 1	0
62.5	75 ± 1	77 ± 3	72 ± 1	63 ± 1	0.00002	80	80	80	71 ± 3	0.0015
p-Value	0	0	0.00002	0.07585		0	0	0.00014	0.01341	

The diameters of the colonies and the decolourized zones are reported as the average of the results on three samples. *p* values were determined for the decolourized zones and colonies diameters, for each source and between different sources.

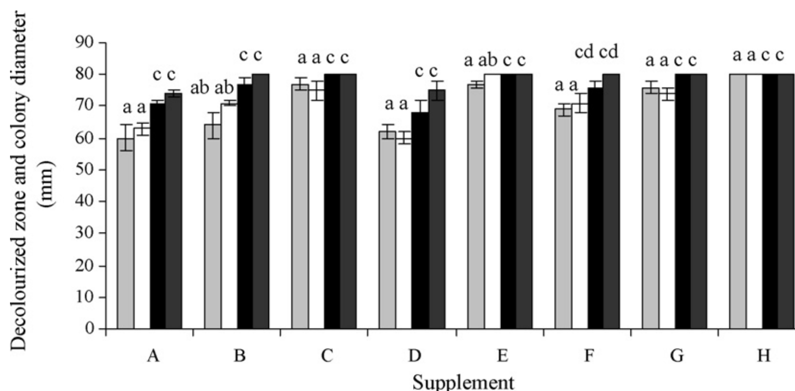

Fig. 1. Decolourized zones diameters ( ), (□) and colonies diameters (■), ( ) for Indigo and Congo red dyes, respectively on media containing natural supplements. (a and c) Indicate average values of decolourized zone and colony diameter, respectively, which are statistically different at $p < 0.05$ for the same dye. (b and d) Indicate average values of decolourized zone and colony diameter, respectively, which are statistically different at $p < 0.05$ between the two dyes.

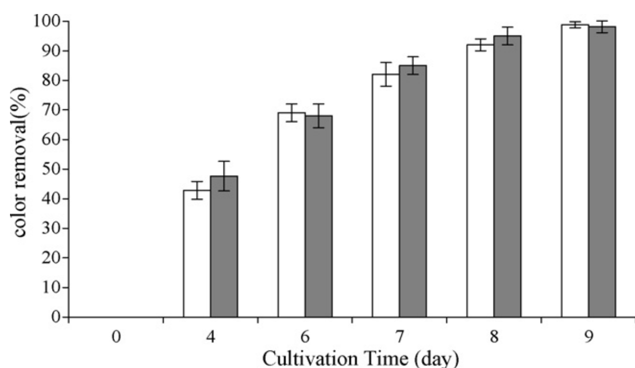

Fig. 2. Colour removal of Indigo (□) and Congo red ( ) in liquid batch cultivation with *Aspergillus alliaceus*. The medium containing (urea 12 mM, glucose 25 mM) for Indigo (150 mg/l) and (yeast extract 8 mM, glucose 25 mM) for Congo red (150 mg/l) supplemented with oak sawdust and wheat bran (2%, w/v). Values shown are the mean of triplicate cultivation experiments.

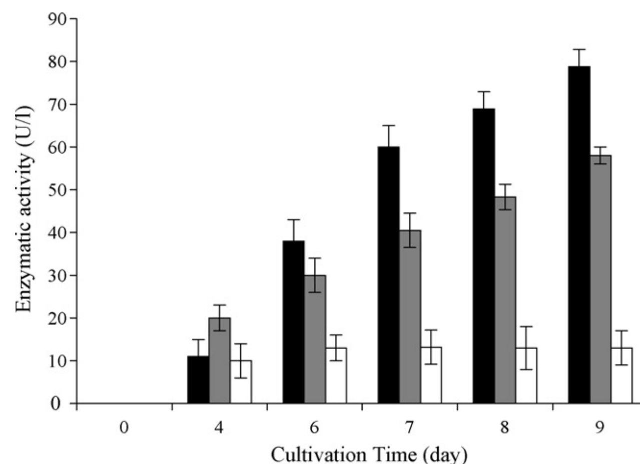

Fig. 4. Laccase (■), LiP ( ) and MnP (□) production by *Aspergillus alliaceus* in liquid medium containing Congo red (150 mg/l), yeast extract (8 mM), glucose (25 mM) and supplemented with oak sawdust and wheat bran (2%, w/v). Values shown are the mean of triplicate cultivation experiments.

decolourization and enzymatic activities profiles for the two dyes are shown in Figs. 2–4.

In liquid medium, the decolourization of the dyes solutions could be due to adsorption by the fungal biomass and biodegradation. When degradation occurred, there was complete removal of the major visible light absorbance peak. Dye adsorption where also evident from the inspection of the mycelial mats, these adsorbing dyes were deeply coloured.

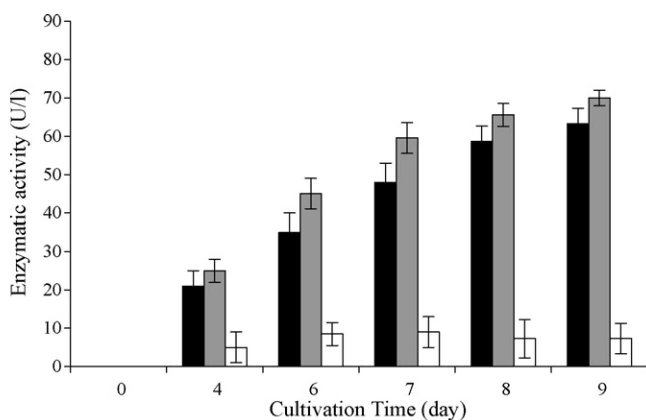

Fig. 3. Laccase (■), LiP ( ) and MnP (□) production by *Aspergillus alliaceus* in liquid medium containing Indigo (150 mg/l), urea (12 mM), glucose (25 mM) and supplemented with oak sawdust and wheat bran (2%, w/v). Values shown are the mean of triplicate cultivation experiments.

Fig. 2 shows that this fungus is able to decolourize these two dyes. In fact, more than 98.6% and 98% of colour removal are obtained, respectively for Indigo and Congo red dyes. As shown in Figs. 3 and 4, when *A. alliaceus* was grown in liquid medium containing optimum carbon and nitrogen sources supplemented with oak sawdust and wheat bran, laccase and LiP activity were detected in the supernatant. The MnP activity is lowly detected. The high activities of laccase (approximately 79 U/l) and LiP (approximately 69.5 U/l) in the culture indicated that these two enzymes may play an important role in the degradation of these dyes.

4. Discussion

The fungi consume and grow on readily available carbon sources at the initial stages of growth and then produce secondary metabolites and extracellular enzymes for biodegradation of dyestuffs at appropriate concentrations of carbon or nitrogen. A primary carbon source such as glucose is necessary for fungal dye decolourization [26,32]. The decolourization activity of *A. alliaceus* was stimulated by all high C-concentrations. Similar results have been reported concerning the role of glucose and other C-sources for fungal decolourization of cotton bleaching with *Phanaerochaete*

chysosporium [33], and black olive mill wastewater decolourization by *Geotichum candidum* [34]. Increasing the C-concentrations in the medium improves fungal decolourization [26]. For decolourization to occur, Swamy and Ramsay [35] showed that a minimum of 0.34 g glucose l^{-1} is required for decolourization. Radha et al. [36] showed that the presence of glucose enhances the rate of decolourization and a concentration of 5.0 g/l was found to be sufficient to achieve the maximum decolourization efficiency of dyes using *P. chrysosporium*. A variety of other sugars can be utilized as sources of carbon and energy, although fungi tend to grow in invasive and filamentous forms on carbon sources that are difficult to utilize such as raffinose or starch [16]. However, Asgher et al. [32] showed that maltose could cause 87% decolourization of solar golden yellow R after 5 days, followed by starch by 86% after 3 days, molasses by 81% after 3 days and fructose by 79% after 1 day, using *Schizophyllum commune IBL-06*. On the basis of these results, glucose seemed to be the most suitable carbon source for decolourization of Indigo and Congo red [25].

In this study, decolourization activity of *A. alliaceus* was stimulated at low nitrogen concentrations of some sources such as Diammonium Tartrate used for Congo red decolourization, and Diammonium Tartrate, peptone and yeast extract used for Indigo decolourization. Only partial decolourization was observed under all high nitrogen growth conditions, suggesting nitrogen-repression of some part of the enzymatic system of this fungus. In fact, the addition of Diammonium Tartrate repressed the decolourization of Congo red only on plates containing high amount of this nitrogen source [37]. The same result was obtained previously by Tatarko and Bumpus [33]. Some N-sources such as Diammonium Tartrate used for Indigo decolourization and urea used for Congo red decolourization inhibited completely the decolourization. With all other sources the decolourization activity is stimulated by high nutrient N-concentrations.

It has been shown that for several species, decolourization is stimulated by high nutrient N-concentrations such as *Bjerkandera adusta* [38], *P. ostreatus* [39,40] and *C. subvermispora* [41]. In contrast, the decolourization activity of *P. chrysosporium* is stimulated at low nitrogen concentrations [42]. Mineralization studies with several dyes have revealed that most of the dyes investigated were degraded extensively only in a certain range of N concentrations [20,24,35,43]. It has been shown that for some fungi, high nitrogen conditions repressed decolourization to some degree. However, nutrient-limitation, especially nutrient nitrogen stimulated enzymes production and nutrient sufficiency repressed enzymes production in most species investigated to date. There are also reports of enzymes production under high nitrogen conditions for some species [37].

The addition of natural supplements (oak sawdust, wheat bran and wheat straw) greatly enhanced ligninolytic enzymes production. In fact, these supplements contain nitrogen, sugars, and organic acids in addition to heavy metals which presented an inducer effect on the ligninolytic enzymes [17,44]. It was showed that by adding certain lignocellulosic residues to the culture medium, especially barley bran similar to this three supplement, ligninolytic enzymes were considerably enhanced. Although the mechanism of induction is unknown, some similar researches propose that organic acids may be responsible for ligninolytic enzymes enhancement [17,24,26].

The ligninolytic activity of *A. alliaceus* grown in liquid medium showed that LiP and laccase were responsible of dye decolourization. Heinzkill and Schinner [45] and Pointing et al. [46] showed that production of these extracelluar enzymes, is strongly affected by the nature and the amount of nutrients especially nitrogen, carbon and microelements. The decolourization can be achieved by two mechanisms, either by adsorption of the dye to the fungal mycelium or by oxidative degradation [47].

Results showed the high ability of *A. alliaceus* to decolourize the two dyes compared with other fungi used in others works. So more than 98.6% and 98% of colour removal are obtained, respectively for Indigo and Congo red dyes. Decolourization of Indigo dye in liquid medium was tested with ligninolytic basidiomycete fungi from Brazil. Decolourization started in a few hours and after 4 days the removal of dye by *Phellinus gilus* culture was 100%, by *P. sajor-caju* 94%, by *P. sanguineus* 91% and by *P. chrysosporium* 75%. They conclude that these fungi will contributed to the decolourization and degradation of effluents containing Indigo dye and their action will reduce the pollutant discharge and toxicity related to the presence of dyes [5].

In previous studies, Congo red was decolourized by *Schizophyllum* sp. *F17* with a maximum decolourization of 91.6% at dye concentration of 15 mg/l [48], then by *P. chrysosporium* [36] and *L. edodes CCB-42* with 92% and 92.3% of decolourization, respectively [49]. Cripps el al. [50] reported that azo dye Congo red was not readily degraded by *P. chrysosporium* and did not appear to be a substrate for lignin peroxydase. However, Ollika et al. [51] reported that Congo red was a substrate for lignin peroxydase. The obtained results are similar to those obtained by Tatarko and Bumpus [33] which confirmed that Congo red is indeed a substrate for lignin peroxydase and it is also readily degraded in liquid and solid agar cultures.

The enzymatic activities of laccase (approximately 79 U/l) and LiP (approximately 69.5 U/l) in the culture indicated that these two enzymes may play an important role in the degradation of these two dyes. The highest laccase activity obtained of growing cells of *F. trogii* in agitated and static batch cultures was 4.61 ± 0.88 and 3.12 ± 0.79 U/ml, respectively. These values were 2.96 ± 0.34 and 2.34 ± 0.46 U/ml for *T. versicolor* [52]. There are several reports on decolourization of textile dyes by the isolated enzymes of white rot fungi. On the other hand, only few reports on the enzymatic decolourization of dye effluents are available. Diluted artificial and real effluents were successfully decolourized by white rot fungi, such as *T. versicolor*, *P. tremellosa*, *Bjerkandera adusta*, *Clitocybula dusenii* and *I. lacteus* [47,53].

5. Conclusion

This study, showed that the decolourization activity of *A. alliaceus,* is strongly affected by the nature and the amount of nutrients especially nitrogen, carbon and microelements on the growth medium. Firstly, this decolourization activity was stimulated at low nitrogen concentrations of some sources such as Diammonium Tartrate used for Congo red decolourization, and Diammonium Tartrate, peptone and yeast extract used for Indigo decolourization. With all other sources the decolourization activity is stimulated by high nutrient N-concentrations. Secondly, this decolourization activity was stimulated by all high nutrient C-concentrations. The addition of natural supplements (oak sawdust, wheat bran and wheat straw) greatly enhanced ligninolytic enzymes production. The detection of enzymatic activities in the culture indicated that laccase (approximately 79 U/l) and LiP (approximately 69.5 U/l) may play an important role in the degradation of these two dyes.

This fungus presents a high ability to decolourize the two dyes. So more than 98.6% and 98% of colour removal are obtained, respectively for Indigo and Congo red dyes. This ability indicated its potential use in antipollution treatments. However, only a better understanding of the mechanisms used by the fungi will allow applying this fungus to the cleaning up aquatic terrestrial environments.

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