Biodegradation of Direct Yellow 28, Red 80, and Blue 71 Azo Dyes Using Fungi of the Genus *Aspergillus*

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Abstract

This work focuses on biodegradation of industrial Direct Yelow 28 (C.I. No 19555, CAS 800-72-9), Direct Red 80 (C.I. No 35780, CAS 2610-10-8), and Direct Blue 71 (C.I. No 34140, CAS 4399-55-7) azo dyes using selected microscopic fibrous fungi of the Aspergillus genus *like* A. candidus*,* A. iizukae *and* A. niger *in aqueous medium. We also attempted to optimize experimental conditions of the biodegradation process and to verify inhibitory effects of dyes at different concentrations on fungi activity.*

Keywords: biodegradation, azo dye, wastewater, Aspergillus

Introduction

Water effluents belong to one of the most significant problem in nowadays world. More than 7,105 tons of textile dyes are produced annually and it is estimated that 15% of the total world production is lost into wastewaters during the dying process [1]. Most of these dyes are toxic to microorganisms [2]. Azo dyes, which represent the largest and most important class of commercial dyes, are hardly biodegradable and their stability is commensurate to the complexity of their molecular structure [3]. Traditional methods for treatment of dye containing wastewaters (such as precipitation, adsorption and/or biodegradation) are often ineffective.

It is known that filamentous fungi produce a wide variety of secondary metabolites, whose play an important role in biodegradation and bioremediation processes [4]. *Aspergillus*, genus of fungi, is a widely studied group producing a diverse spectrum of beneficial metabolites whose attract immense interest due to their potential on the field of biotechnological applications as, e.g., treatment of industrial waste water originating from dyeing of textiles [5]. A wide variation in metabolism, reproductive strategies, and survival mechanisms are found among members of genus *Aspergillus*, rendering them not only as resilient and diverse microorganisms, but also as important organisms for biological study.

Materials and methods

Microscopic fungi

The basis for the degradation of dyes was previously proven adaptability of applied *Aspergillus* isolates to organic substances and other pollutants of organic origin. A tolerance to organic substances in lagoon sludge was utilised in experimental degradation of dyes under study.

Strains of the *A. iizukae* LA-3, *A. niger* LA-18 and *A. candidus* LA-21 filamentous fungi (Fig. 1) were isolated from sludges originating of refinery production and regeneration of waste lubricating oils as they were deposited into local Ostrava Lagoons [6]. Due to a very high contamination caused by organic sludge, those isolates have been evaluated as very adaptable microorganisms with good chance regarding remediation [7]; therefore, they were selected to verify the biodegradability of aqueous solutions azo dyes studied.

Preparation of samples of model dyes

For the experimental use, commercial Direct Yellow 28, Direct Red 80 and Direct Blue 71 (Synthesia a.s., Czech Republic, Fig. 2a-c) were dissolved in hot 50% pyridine and filtered off. After cooling, products were precipitated with addition of methanol and solids were washed with methanol. Yields of recrystallizations were about 25% and contents of dyes in samples have reached about 90% (determined by elemental analysis). Structures were also confirmed using mass spectroscopy (Fig. 3a-c). Identities of samples have been tested using thin layer chromatography on Silufol with a 254 nm UV filter as stationary phase and 2/1 (v/v) n-propanol/ammonia mobile phase. No impurities were detected in the samples after recrystallization.

Sample 0.035 $g.L^{-1}$ of yellow dye in distilled water has exhibited absorbance maximum at 395 nm, 0.0311 $g.L^{-1}$sample of red has absorbance maximum at 530 nm, and blue 0.027 $g.L^{-1}$ has shown absorbance maximum at 585 nm.

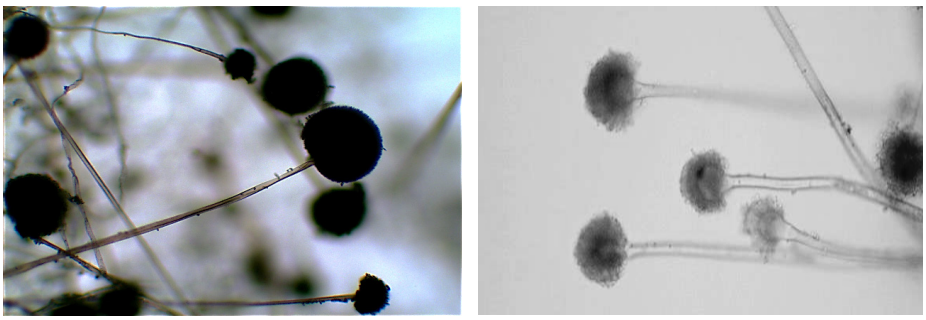

Fig. 1. Aspergillus niger LA-18 and Aspergillus iizukae LA-3 (Olympus CX41, magnified 400x)
Rys. 1. Aspergillus niger LA-18 i Aspergillus iizukae LA-3 (Olympus CX41, powiększenie 400x)

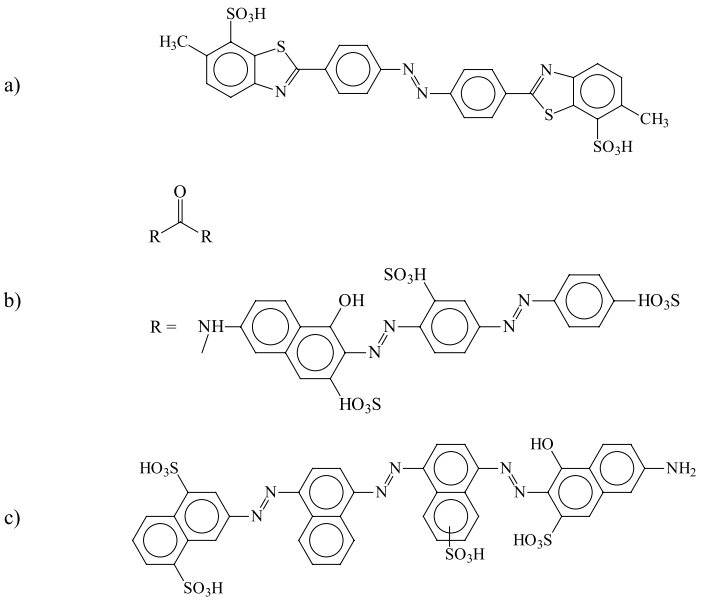

Fig. 2. a) Direct Yellow 28, M = 637 g.mol^{-1}, b) Direct Red 80, M = 1241 g.mol^{-1}, and c) Direct Blue 71, M = 942 g.mol^{-1}
Rys. 2. a) Direct Yellow 28, M = 637 g.mol^{-1}, b) Direct Red 80, M = 1241 g.mol^{-1} oraz c) Direct Blue 71, M = 942 g.mol^{-1}

Fungal biodegradation

Spores of *Aspergillus* fungi obtained as new isolates from soils contaminated with a high content of organics were inoculated into dyes solutions by following routine: all selected isolates of *Aspergillus* fungi were cultivated on Petri dishes with Sabouraud dextrose agar (M063, HiMedia Laboratories, Mumbai, India) at first. After 10 days culturing, spores were separated from strongly spore-forming cultures and inoculated into Erlenmeyer flask containing dye solution. Occasionally, samples were agitated during experiment.

Biodegradation experiments were performed in the form of static samples culturing for 10 days at 25°C.

After 10 days of biodegradation, samples were filtered (KA-3M, Filpap, Czech Republic), centrifuged for 15 min at 4400 rpm and then UV/vis absorption spectra were measured. A reference sample of the dye solution without the presence of fungi was also measured for comparison.

MS and UV/vis absorption spectrometry

ESI-MS spectra (Fig. 3a-c) were measured on a mass spectrometer with a hybrid QqTOF analyzer (MicrOTOF-Q, Bruker Daltonics, Germany) in the range of 50–1500 m/z when recording negative ions. Before measurement, the instrument was externally calibrated using sodium formate clusters. Dyes were dissolved in acetonitrile/water (1/1). Capillary voltage was 4.5 kV, drying gas temperature was 200°C, the flow rate and nitrogen pressure were 4 L.min^{-1} and 0.4 bar.

Absorption spectra (Fig. 3d) were measured on a Hewlett Packard 8453 UV/vis spectrophotometer (Agilent Technologies, USA) in a 1 cm path length quartz cuvette. Molar absorption coefficients of the yellow dye (ε(396) = 15500 L.mol^{-1}.cm^{-1}), red (ε(528) = 24600 L.mol^{-1}.cm^{-1}), and blue (ε(586) = 50300 L.mol^{-1}.cm^{-1}) were determined by measuring five concentration standards in distilled water.

Results and discussion

Direct Yellow 28 was decoloured with *A. niger* fungi, which was able to bleach the dye by 93% after 10 days. Direct Red 80 and Direct Blue 71 dyes were most effectively bleached by the *A. candidus* fungi, concentration of Red 80 was reduced by 95% after 10 days and the Blue 71 by 84%. Results on fungi biodegradations of dyes after 10 days are shown in Table 1.

Figure 4 shows UV/vis absorption spectra of samples of dyes as for the beginning of the experiment as also after 10 days of action of selected fungi strains. It has been found that fungi under study have bleached dyes, but they produced metabolites absorbing in the visible region at the same time.

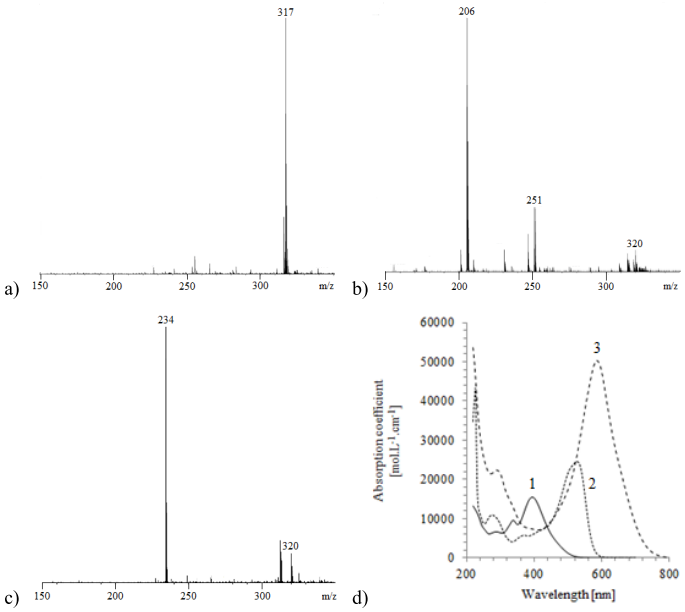

Fig. 3. ESI mass spectra for the negatively charged ions: a) Yellow 28 m/z: 317 $[M - 2Na]^{2-}$ (100%), b) Red 80 m/z: 320 $[M - 4Na]^{4-}$ (8%), 251 $[M - 5Na]^{5-}$ (25%), 206 $[M - 6Na]^{6-}$ (100%), c) Blue 71 m/z: 320 $[M - 3Na]^{3-}$ (12%), 234 $[M - 4Na]^{4-}$ (100%) and (d) UV/vis absorption spectra of yellow (1), red (2) and blue (3) in distilled water

Rys. 3. Widma masowe ESI dla ujemnie naładowanych jonów: a) Żółty 28 m/z: 317 $[M - 2Na]^{2-}$ (100%), b) Czerwony 80 m/z: 320 $[M - 4Na]^{4-}$ (8%), 251 $[M - 5Na]^{5-}$ (25%), 206 $[M - 6Na]^{6-}$ (100%), c) Niebieski 71 m/z: 320 $[M - 3Na]^{3-}$ (12%), 234 $[M - 4Na]^{4-}$ (100%) i (d) widma absorpcyjne UV/vis koloru żółtego (1), czerwonego (2) i niebieskiego (3) w wodzie destylowanej

Tab. 1. Results on fungi biodegradations of dyes under study after 10 days (recovery in %)

Tab. 1. Wyniki biodegradacji grzybów badanych barwników po 10 dniach (odzysk w%)

	Biodegradation[a)]					
	Direct Yellow 28		**Direct Red 80**		**Direct Blue 71**	
Fungi strain	Absorbance – Dye removal [%]		Absorbance – Dye removal [%]		Absorbance – Dye removal [%]	
Reference sample	1,49986	**0**	1,09536	**0**	1,45620	**0**
A niger LA-18	0,10507	**93,0**	0,24588	**77,6**	0,43538	**70,1**
A iizukae LA-3	0,71067	**52,6**	0,31495	**71,3**	0,24938	**82,9**
A candidus LA-21	0,47646	**68,2**	0,05136	**95,3**	0,22654	**84,4**
Mixture of fungi used	0,28851	**80,8**	0,23316	**78,7**	0,31800	**78,2**

[a)] average value

The most well-known fungi in terms of degradation of dyes are the ligninolytic fungi, which produce a variety of active enzymes degrading the various textile dyes due to nonspecific metabolic systems [8–9] (fungi producing many active enzymes due to their non-specific enzyme systems). Since 1999, it has been shown that laccases and azo-reductases represent enzymes significantly degenerating azo dyes. Other important enzymes include phenolic oxidases, peroxidases, hydroxylases and a variety of azo-dye reductases [10].

The activity of microscopic fungi of the *Aspergillus* genus in the degradation of textile azo dyes has not been sufficiently explored due to the number of representatives of this genus. Although number of works have been published to verify the biodegradation activity of several representatives, in particular *A. niger*, *A. foetidus*, *A. oryzae* and *A. ochraceus* [11–16], the activity of microscopic fibrous fungi *A. candidus* and *A. iizukae* has not yet been published. In this respect, an interesting study by [17], which examined the biosorption ability of *A. niger* and *A. terreus*, was to bind the colour components and metals from pigments, as well as their decolorizing capabilities. When testing the decolourization of coloured solutions with *A. niger*, the percentage of decolourization of the samples was approximately 30%, while in the biodegradation process with the fungi *A. terreus*, after 336 hours, the solution was discoloured by up to 98%. However, even in other studies, it was confirmed that the rate of degradation is the most intense within 10 days [18], after 240 hours (10 days) it significantly decreases. After 336 hours, significant spectral changes leading to toxic conversions were found in the dye molecules, as confirmed by acute toxicity and mutagenicity tests (up to ten-fold increase in the degree of toxicity of colorant residues) [19–20].

10-Days biodegradation has also proven itself in our experiments. In the check results of continuing biodegradation after 20 days in simulated samples of azo dyes, significant changes in the colour of monitored solutions (Fig. 4) were found; they were caused due to production of various pigments produced by the fungi of the *Aspergillus* genus as intermediate metabolites during their growth [21]. It is known that the *Aspergillus* genus representatives produce various coloured pigments including melanins [22] and it has been shown previously that increased pigment production is close-

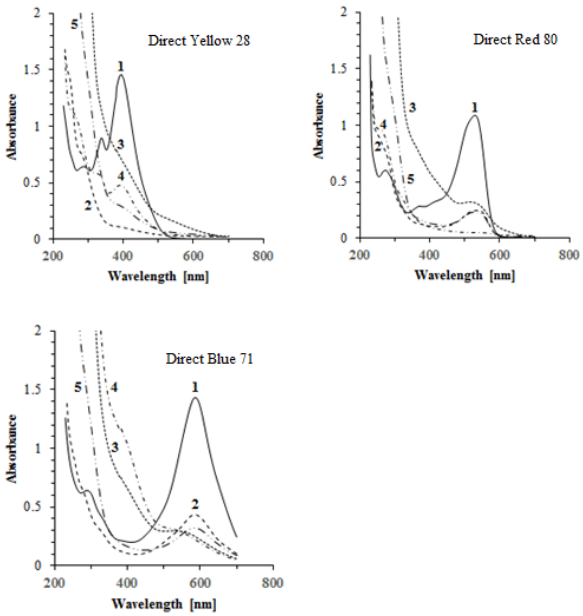

Fig. 4. UV/vis absorption spectra for samples of dyes containing tested fungi after 10 days. Curve 1: reference sample of dye, curve 2: *Aspergillus niger*, curve 3: *Aspergillus iizukae*, curve 4: *Aspergillus candidus*, curve 5: mixture of all fungi

Rys. 4. Widma absorpcyjne UV / Vis dla próbek barwników zawierających badane grzyby po 10 dniach. Krzywa 1: próbka odniesienia barwnika, krzywa 2: *Aspergillus niger*, krzywa 3: *Aspergillus iizukae*, krzywa 4: *Aspergillus candidus*, krzywa 5: mieszanka wszystkich grzybów

ly related to the adaptation of fungi to stress conditions in the environment [23]. Fungi isolates selected in our experimental work produce related pigments. A dark brown pigment aspergillin was detected in the *A. niger* check culture filtrate after 20 days as well as in the case of the *A. iizukae* fungi when the brownish soluble pigment iizukine [24] coloured the samples. Although *A. candidus* is also closely related to the darkly pigmented group of *Aspergillus* (including *A. niger*) [25], it is known as the white-spored species. In this strain the white pigment production is limited, so its decolorizing activity increased slightly after 20 days (for example in the yellow dye to 92%) because it was not affected by the production of dark metabolites.

Conclusion

Azo dyes, the largest class of commercially available textile synthetic dyes, are often used not only for textile and leather dyeing, but also in drugs and cosmetics industry. Their occurrence in wastewater, particularly in Asian regions, is alarming today and affects life forms at all levels.

It turns out that the physicochemical methods of industrial wastewater treatment do not remove these dyes efficiently. In addition, these dyes are not readily degraded by microorganisms, although microbial degradation involving wastewater decolourization has gained more attention lately, primarily due to the environmentally friendly and less costly nature of the process as a whole.

The use of *Aspergillus* microscopic fibrous fungi of the *Aspergillus genus* in bioremediation of textile wastewater is a suitable alternative to microbial degradation. When selecting a suitable degrading microorganism, it is possible to degrade the tested azo dyes after 10 days with very high efficiency. Based on the experiments, it was found that the best degradable dye is Direct Red 80, which was degraded by the fungi *A. candidus* by 95%. *A. niger* degraded Direct Yellow 28 by 93%, and Direct Blue 71 was degraded after 10 days up to 84%. When a joint consortium was applied, the efficacy was lower due to the co-production of secondary metabolites by fungi applied.

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Biodegradacja barwników azowych Direct Yellow 28, Red 80 i Blue 71 przy użyciu grzybów z rodzaju Aspergillus

Niniejsza praca koncentruje się na biodegradacji przemysłowych barwinków azowych Direct Yelow 28 (CI nr 19555, CAS 800-72-9), Direct Red 80 (CI nr 35780, CAS 2610-10-8) i Direct Blue 71 (CI nr 34140, CAS 4399 -55-7) przy użyciu wybranych mikroskopijnych włóknistych grzybów z rodzaju Aspergillus*, takich jak* A. candidus*,* A. iizukae *i* A. niger *w środowisku wodnym. Przeprowadzono próby optymalizacji warunków eksperymentalnych procesu biodegradacji i weryfikacji hamującego wpływu barwników o różnych stężeniach na aktywność grzybów.*

Słowa kluczowe: biodegradacja, barwnik azowy, ścieki, Aspergillus