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Enhanced decolorization and biodegradation of acid red 88 dye by newly isolated fungus, *Achaetomium strumarium*

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ABSTRACT

Acid red 88 dye degradation efficiency of newly isolated filamentous fungus, *Achaetomium strumarium* were investigated. Molecular studies of 23S rRNA sequence data confirmed the phylogenetic clade relationship of the isolate with members of the same genus, *Achaetomium*. *Achaetomium strumarium* decolorized (99%) of 10 mg L^{-1} of acid red 88 dye at pH (4), biomass dose (2000 mg) and temperature (40 °C) within 96 h. Further studies revealed that decolorization was enhanced with the addition of calcium salts in the reaction medium resulting in maximum amount of dye adsorbed (35.55 mg g^{-1}). The experimental data showed the best goodness of fit when subjected to Temkin isotherm model (R^2 = 0.985) in comparison with Freundlich and Langmuir isotherm models (R^2 = 0.883 and 0.688) respectively. The adsorption mechanism followed pseudo-second order kinetic model (R^2 = 0.997) indicating the influence of the AR88 dye molecules and fungal biomass. Enzymes analysis revealed significant inductions and role played by NADH-DCIP reductase and laccase in the asymmetric cleavage, dehydroxylation, and desulfonation of AR-88 dye. Metabolites of the acid red 88 dye after degradation were analyzed using UV–vis spectroscopy, FTIR, HPLC and GCMS. The GCMS analysis revealed the production of three intermediates; naphthalen-2-ol, sodium naphthalene-1-sulfonate and 1,4-dihydronaphthalene. Possible metabolic fate pathway for the degradation of AR88 dye by *A. strumarium* was proposed. The results obtained from toxicity studies revealed the AR-88 dye detoxification efficiency of *Achaetomium strumarium* and hence, in its myco-transformation.

1. Introduction

Azo dyes are constantly and widely applied directly or indirectly by pharmaceuticalpaper printing pulp making food leather dyeing and petroleum industries [1]. Crass pollution especially in developing nations arises from increasing applications of these class of dyes. Textile and dyestuff manufacturing industries account largely for the indiscriminate release of the dye into the environment [2]. Commercial synthetic dyes azo dyes inclusive represents up to 70% of the total textile dyestuffs used in industry [3]. Azo dye toxicity to plants human and animals causes grave environmental concerns owing to their characteristic brilliance color carcinogenic potentials and recalcitrance. Azo dyes recalcitrant nature is due to the possession of highly stable reactive azo bond (—N═N—) in their structures (heterocyclic and aromatic) [1]. High quantities of dyes are dumped into the environment as a result of rapid industrialization and urbanization. Up to half the amount of the original dye is lost to wastewater during dyeing and dye fixation processes [4]. The significant increases in oxygen demand (dissolved chemical and biological) and metals ions is largely caused by the improper discharge of dyes in water bodies [5]. Therefore to reduce the effect of these discharges it is very necessary though arduous to treat the dye effluents. Only few of several physicochemical

Abbreviations: UV–vis, Ultra Violet Visible spectrophotometer; FTIR, Fourier Transform Infrared spectroscopy; GCMS, Gas Chromatography Mass Spectrometry; ADMI, American Dye Manufacturer's Institute; HPLC, High-Performance Liquid Chromatography; APHA, American Public Health Association; NADH, Nicotinamide adenine dinucleotide; DCIP, Dichlorophenol Indophenols; R, Correlation coefficient; R^2, Coefficient of determination; S.E.M, Standard Error of Means; q_e, Amount of dye adsorbed (mg g^{-1}); b_T, The adsorbent at the equilibrium; *qt*, Amount adsorbed by the adsorbent at any time (mg g^{-1}); C_e, Concentration of the indigo dye at the equilibrium (mg L^{-1}); C_o, Initial concentration of the indigo dye (mg L^{-1}); n, The order of adsorption with respect to the effective concentration of the adsorption active sites present on the surface of the adsorbent; R, The universal gas constant (8.314 J K^{-1} mol^{-1}); T, The absolute temperature at 298 (K); b_T, Temkin isotherm constant,; A_t, Temkin isotherm equilibrium binding constant (L g^{-1}); K_F, The Freundlich equilibrium constant (mg g^{-1} (mg L^{-1}) $^{-1/n}$); n_F, The Freundlich exponent (dimensionless); K_L, Langmuir isotherm constant (L mg^{-1}); B, Heat sorption constant (J mol^{-1}); Q_o, Maximum monolayer coverage capacity (mg g^{-1}); k_1, The pseudo-first-order rate constant (min^{-1}); k_2, The pseudo-second-order rate constant (g mg^{-1} min^{-1}); *t*, Contact time (min)

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decolorization technologies have been found worthy by the textile waste managers [6]. In recent years several physicochemical methods have been employed in the removal of dye from textile effluent but these methods have been certified unsuitable and uneconomical due to the production of large amounts of toxic sludge aromatic amines toxic substances and secondary waste products [7]. These degradation intermediates viz: phenols benzene naphthalene and aromatic amines very toxic and recalcitrant. Also Myslak and Bolt [8] reported the carcinogenic and mutagenic nature of aromatic amines produced after biodegradation of azo dyes. Proper disposal challenges arise as a result of accumulated concentrated sludge [9]. The general drift in research nowadays is to improve the natural degradation capacity using indigenously isolated organism. Azo dye biodegradation research focus has drifted recently towards the use of algae fungi yeasts inclusive and bacteria. Despite algae and bacteria potency in azo dye degradation due to their ease of multiplication in record time high biochemical activity great adaptability and extensive distribution [10] their biochemical activities are sometimes disrupted by azo dye metabolites (such as aromatic amines) after degradation. Azo dye detoxification and decolorization capacity of microorganisms have been reported by an ample number of researchers [11,12]. Alternatively fungal biomass use as biosorbents has proven to be more eco-friendly cheap and sustainable. Filamentous fungi dissipate great adaptability and efficiency in the mineralization of these aromatic compounds through adsorption/biodegradation [13]. The role played by dye molecular structure in the adsorption mechanism of azo dyes by fungi cannot be over emphasized [14]. Filamentous fungi break down complex dye molecules by secreting metabolic enzymes after bonding with the dye molecules effectively [15]. Due to their massive biomass production a great surface to cell ratio solid-liquid separation and extensive hyphal development quite a number of filamentous fungal species have been explored for azo dye biodegradation [16]. Filamentous fungi secrete diverse extracellular non-specific ligninolytic enzymes which confer on them the capacity to degrade a wide range of environmental pollutants such as pesticides pharmaceutical waste products aromatic hydrocarbons dyes phenols polychlorinated biphenyls and dioxins [5]. Adsorption and degradation mechanisms are deployed by most filamentous fungi in the decolorization of various dyes [15]. Therefore further exploration study research and exploitation at discovering new fungal strains capable of degrading azo dyes efficiently are still germane to the huge and commercial application of bioremediation. In this present study acid red 88 dye was chosen as the preferred adsorbate because of its vast application in dyeing textile fabrics silk nylon wool and leather and the carcinogenic nature of its biotransformed products. There are fewer reports on biodegradation of acid red 88 dye by filamentous fungi [17]. However this is the first report of isolation and characterization of *Achaetomium strumarium* from Nigerian soil. Furthermore there is no report of biodegradation of acid red 88 dye by a filamentous fungus *Achaetomium strumarium*. This study is aimed at investigating the potentials of a newly isolated fungus *Achaetomium strumarium* in the removal of acid red 88 dye sequel to previous decolorization experiments done by Bankole et al. [18]. There is also no detailed study of adsorption isotherms kinetics and enzyme mechanisms of acid red 88 dye degradation by filamentous fungus. Biodegraded products were characterized using Ultra-Violet visible spectroscopy (UV–vis) Fourier transform infrared spectroscopy (FTIR) Gas chromatography mass spectroscopy (GCMS) and High performance liquid chromatography (HPLC). Adsorption isotherms (Langmuir Freundlich and Temkin) and kinetics (pseudo first-order and pseudo second-order) studies were equally carried out to understand the equilibrium standards of acid red 88 dye biodegradation. Toxicity studies were done to evaluate the detoxified status of acid red 88 dye after biodegradation.

2. Materials and methods

2.1. Organism culture condition and molecular characterization

The fungus was isolated from the soil around Itoku area (7°48′09.62″N; 4°28′06.63″E), Abeokuta Ogun State Nigeria [18]. The location is one with a large number of small scale textile dye industries and practitioners. The isolate was identified to the species level through DNA analyses of the 23 rRNA gene. The fungus was initially maintained and periodically subcultured using Potato Dextrose Agar (PDA Oxoid UK) at ambient temperature (28 ± 2 °C). Genomic DNA was extracted from fresh mycelia (30 mg wet weight) collected from 100 ml potato dextrose broth using the Zymo Research DNA Extraction kits. The ultrapure extracted fungal genome was later sent to Laragen IncorporatedCulver city California USA for DNA sequence analysis. Free Basic local Alignment Search Tool (BLASTn) service available on the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/) was used in evaluating the homologous identities and similarities with previous submissions. The data were thereafter deposited in the GenBank. Phylogeny was evaluated using the Neighbor-Joining method [19] with a branch length (0.12922884). With a total of 1949 positions in the final dataset Maximum Composite Likelihood method [20] was used in determining the phylogenetic distances between 30 nucleotide sequences. Molecular Evolutionary Genetics Analysis (MEGA7) software was used for the analyses [21].

2.2. Dye and chemicals

Purchase of HPLC grade methanolaniline-2-sulfonic acid riboflavin-2-sulfonic acid and 2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were from Kemira ChemicalsBradford United Kingdom. All other chemicals and solvents of guaranteed reagent garde were purchased from Sisco Research Laboratory India. The azo dye C.I. Acid Red 88 (Colour index number: 15620 75% purity chemical formula: $C_{20}H_{13}N_2NaO_4S$ molar mass: 400.38 g mol-1) (Fig. 1) commonly used by local textile processing industries in Nigeria was procured from Sigma Aldrich Chemicals (St. Louis MO USA). Procurement of media and other chemicals were from Oxoid Laboratories UK. All chemicals used were of high analytical grade with no prior purification process done.

2.3. Decolorization experiments

Acclimatization was performed by gradually exposing the fungus to the increasing concentration of acid red 88 dye 10, 20, 30, 40, 50, 60, 70, 80 mg L^{-1} at 30 °C in static condition to determine the decolorization potency. The experiments were done in three sets in 250 ml Erlenmeyer flasks of mineral salts medium (g L^{-1}) containing $CaCl_2{\cdot}2H_2O$ (0.10), Fe (NH_4) citrate (0.01), Na_2HPO_4 (3.6), $(NH_4)_2SO_4$ (1.0), KH_2PO_4 (1.0), $MgSO_4$ (1.0), supplemented with 0.05% (w/v) of yeast extract, 2.8 mM glucose and acid red 88 dye (10 mg L^{-1}) at 29 °C and pH 4 in static conditions [22]. Aliquots (2 ml) of the culture media was withdrawn at different time intervals, centrifuged at 10000 rpm for 10 min to separate the fungal biomass. The decolorization performance was examined spectrophotometrically (Hitachi U-2800 ; Japan) at the maximum absorption wavelength (λ_{max} = 505 nm) and percentage decolorization was calculated as follows:

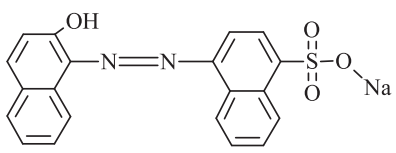

Fig. 1. Chemical structure of dye C.I. Acid Red 88 (CI 15625; λ_{max} = 505 nm).