



Oxidative Decolorization of Direct Blue 71 Azo Dye by *Saccharomyces cerevisiae* Catalyzed by Nano Zero-valent Iron

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Authors' contributions

This work was carried out in collaboration between all authors. Author NAHF designed the study, wrote the protocol, performed the biodegradation experiments and interpreted the data. Author AZAA performed the chemical analysis and interpreted the data. Author IMI identified the yeast strain. Author SAS synthesized and characterized the zero-valent iron nanoparticles. All authors managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

The textile industries wastewater is one of the most serious sources of environmental pollution due to presence of the textile dyes. In the present study, five bacteria and three fungi were isolated on Mineral Salt Medium (MSM) supplemented with 200 mg/l of Direct Blue 71 azo dye. The most active isolate was identified by 18S-rRNA gene sequencing method as *Saccharomyces cerevisiae*. The decolorization and degradation of Direct Blue 71 azo dye by *S. cerevisiae* was investigated in

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presence and absence of nano-scaled zero-valent iron (NZVI). The metabolic pathway of the dye degradation was elucidated using GC/MS analysis to identify the metabolites. This procedure was supported by enzymes assay and bioinformatics analysis. Dye-decay curves showed 96% dye removal after 72 hr. in absence of the catalyst; while it was 100% after 48 hr. in presence of 0.1% NZVI. High efficiency to decolorize Direct Blue71 enables this yeast to be used in biological treatment of industrial effluent containing such azo-dyes.

Keywords: *Saccharomyces cerevisiae*; direct blue 71; azo-dye; decolorization; biodegradation; Nano Zero-valent Iron (NZVI).

1. INTRODUCTION

Textile industry is one of the major industries in the world that provide employment with no required special skills and plays a major role in the economy of many countries. A large amount of liquid wastes are produced daily. It was about 1,000-3,000 m³ of water is spilled out after processing of about 12-20 tons of textiles/ day) [1]. The largest group of colorants used in industry are the azo dyes, which is characterized by presence of one or more azo bond (–N=N–) and resistance to biodegradation [2]. Some of the degradation products are toxic such as aromatic amines that cause mutagenic to the human hepatoma cell line [2] and lymphocytes and in HepG2 [3].

The iron nanoparticle technology has got more attention for its effective application in azo dye bioremediation [4]. The nano-scaled zero-valent iron (NZVI) was used to destruct halogenated hydrocarbons and organochlorine pesticides and organic dyes [5,6]. Promising results for in situ remediation were described [7]. Current research is moving towards applications of nano- sized particles, such as zero-valent iron, in azo dye bioremediation.

Therefore, the aim of this work was to study the microbial degradation of the azo dye Direct Blue 71 by a local microbial isolate catalyzed by NZVI. Furthermore, to identify the proposed biodegradation pathway of the direct blue azo dye by the isolated microorganism.

2. MATERIALS AND METHODS

2.1 Azo Dye and Chemicals

Direct blue 71 Azo dye (DB71) (1,5-Naphthalenedisulfonic acid, 3-[[4-[[4-[(6-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-6-sulfo-1-naphthalenyl]azo]-1-naphthalenyl]azo]-, tetrasodium salt) and all chemicals used in the enzyme assays were obtained from Sigma-Aldrich. Dye stock solution (1 g l⁻¹) was prepared in distilled water and used in all experiments.

2.2 Sample Collection

Effluent contaminated soil was collected from the upper (20–30 cm) layer of vegetable field behind cluster of small scale dyeing and printing units Located at Shoupra (Kaliobia, Egypt).

2.3 Isolation of Dye Biodegrading Isolates

The microbial isolates were isolated by pouring plates methods using Mineral Salt Medium (MSM) containing (% w/v): (NH₄)₂SO₄ (0.25), yeast extract (0.25), KH₂PO₄ (0.5), MgSO₄·7H₂O (0.05), CaCl₂·2H₂O (0.013), pH 6.5 (16). The medium was supplemented with 200 mg l⁻¹ of Direct blue 71 azo dye.

2.4 Identification of the Yeast Isolate

The most active isolate, that showed highest decolorization efficiency, was selected and characterized based on cell morphology and identified by 18S-rRNA gene sequencing method. Genomic DNA was extracted from pure cultures of the yeast isolate using the DNeasy plant mini kit (Qiagen, CA, USA) according to the manufacture's instructions. The primers used to amplify the 18S ribosomal RNA gene were ITS3F (5'- GCATCGATGAAGAACGCAGC -3') and ITS4R (5'-TCC TCC GCT TAT TGA TAT GC-3') (17). DNA amplification was performed using 20 ng of DNA template 250 mM each dNTPs [dATP, dCTP, dGTP, dTTP], 25 pmol each primer, 2.5 mM MgCl₂ 10 µl of 5x PCR buffer and 2.5 U Taq polymerase (promega) and added up to total volume of 50 µl ddH₂O. The reactions were performed in an automatic thermal cycler (GeneAmp1 PCR System 9700, Perkin-Elmer) under the following conditions: initial denaturation at 94°C for 3 min; 35 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min; final extension at 72°C for 7 min. An aliquot of 10 µl PCR product were analyzed on 1% agarose gel. The purification of PCR product was done using high pure PCR purification kit (Qiagen). The DNA sequencing was performed using ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, USA) in

conjunction with ABI PRISM (310 Genetic Analyzer). Approximately 380 bases of nucleotide sequence were obtained. Sequence data was aligned and compared with other rDNA sequences in the GenBank using the NCBI basic local alignment search tools (BLAST-n) program (<http://www.ncbi.nlm.nih.gov/BLAST>).

2.5 Preparation of the Nano-zero Valent Iron (NZVI)

The NZVI was prepared by the reduction of an aqueous ferrous sulfate by using sodium borohydride (NaBH_4). Ten g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were dissolved in 100 ml of 30% ethanol and 70% deionized water. The pH value was adjusted to 6.8 with 3.8 N $\text{NaOH}(\text{aq})$. About 1.8 g of NaBH_4 powder was added incrementally to the mixture, allowing the forming to subside between increments. After addition of the NaBH_4 powder, the mixture was stirred for about 20min and then the solid particles were separated by centrifugation at $10000 \times g$ for 15 min. and washed three times with 25 ml portions of absolute ethanol to remove all of the water. This washing process is probably the key step of synthesis since it prevents the rapid oxidation of zero valent iron nanoparticles. The synthesized nanoparticles were finally dried in oven at 50°C overnight. For storage, a thin layer of ethanol was added to preserve the nano iron particles from oxidation.

2.6 Characterization Techniques of NZVI

An X-ray measurement of various mixed solids was carried out using a Bruker D8 advance diffractometer (Germany). The patterns were run with Cu Ka radiation at 40 kV and 40 mA with scanning speed in 2θ of 2°min^{-1} . TEM image and selected-area electron diffraction (SAED) was taken with a JEOL JEM-2000 EX model transmission electron microscope, using an accelerating voltage of 100 kv. The surface characteristics of various solid catalysts, namely, the specific surface area (S_{BET}), total pore volume (V_p) and mean pore radius (\bar{r}) were determined from nitrogen adsorption isotherms measured at -196°C , using Nova 2000, Quanta Chrome (commercial BET unit). Before undertaking such measurements, each sample was degassed under a reduced pressure of 10^{-5} Torr for 2 h at 200°C .

2.7 Decolorization Experiments

All decolorization experiments in this study were carried out as follows unless otherwise stated:

Eighty ml of MSM supplemented with 1% glucose was inoculated with a loop of the microbial isolate incubated for one day in shaking flasks at 150 rpm and at $28 \pm 2^\circ\text{C}$. Twenty ml of a sterile Direct blue 71 stock solution (1 g l^{-1}) was added (to give a final dye concentration of 200 mg l^{-1}) into the 24 hr old culture and incubated at 28°C under shaking condition. After decolorization, the biomass was removed by centrifugation ($4000 \times g$, 20 min) and the absorbance of supernatant was measured at 587 nm. by using Shimadzu UV spectrophotometer (Shimadzu UV 1800, Japan). The decolorization was expressed in terms of percentage and calculated as follows:

$$\text{Decolorization (\%)} = \frac{[(\text{initial absorbance}) - (\text{observed absorbance})]}{(\text{initial absorbance})} \times 100$$

The control flasks were prepared containing all medium components without inoculum. The changes in the absorption spectrum of both treatment and control in the visible range (400–800 nm) were recorded. All the decolorization experiments were carried out in three replicates and the data average was recorded.

2.8 Effects of NZVI on Direct Blue 71 Decolorization

Different Nano zero-valent iron weights ranging from 0.25 to 2 g l^{-1} were added into liquid culture of the yeast isolate to determine their effects on the decolorization of Direct blue 71 dye. After 24 hr of incubation, the dye was added to a final concentration of 200 mg l^{-1} . The flasks were incubated as mentioned above and the decolorization was determined after intervals of 24, 48 and 72 hr. Control flasks were prepared from the MSM with the dye and the same weight ranges of the NZVI (without the yeast). All experiments were carried out in three replicates and the data average was recorded.

2.9 Optimization of the Biodegradation Parameters

To study effect of static and shaking (150 rpm) conditions, the decolorization experiments were conducted and measured as mentioned above. The same trend was followed up to determine effect of pH (5-8), incubation temperature (25°C , 28°C , 32°C , 37°C and 40°C) and the initial concentration of Direct blue 71 dye ($50, 100, 200, 300, 400$ and 500 mg l^{-1}). All experiments were

carried out in three replicates and the data average was recorded.

2.10 Extraction of Degradation Products of Direct Blue 71

The degradation products were extracted from the culture supernatant by an equal volume of ethyl acetate. The extract was evaporated under vacuum to 2 mL final volume.

2.11 GC/MS Analysis

GC-MS analysis was carried out by using HP-5890 GC equipped with HP-5972 mass spectrometer. The separation conditions were; inlet temperature (280°C), mobile phase (helium), flow rate (1 ml min⁻¹), oven temperature program (initial temperature 80°C, 10°C min⁻¹, up to 280°C for 10 min), HP-5MS column (30 m X 0.25 mm ID) and MS detector temperature (300°C). The obtained mass spectra were analyzed by Wiley7N mass library.

2.12 Enzyme Assays

Peroxidases, catalases, azoreductase and catechol-1,2-dioxygenase were determined in the culture supernatant of yeast grown in each of the following media: LB medium (control medium), MSM supplemented with the dye in presence and absence of NZVI (catalytic and non-catalytic conditions). One unit of peroxidases was expressed as the enzyme amount that catalyzes production of one milligram of purpurogallin in 20 seconds at 20°C at pH of 6.0 [8]. One unit of catalase is the enzyme amount that decomposes 1 pMol of hydrogen peroxide/min./ml culture [9]. Di-oxygenase activity was determined as described by Hegeman [10]. Azoreductase was determined by the method described by Montira and Sukallaya [11]. One unit of azoreductase was expressed as the enzyme amount that catalyzes degradation of one μmol of methyl red dye per min.

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of the Azo-dye Decolorizing Yeast

Five bacterial, three molds and two yeast isolates were isolated and purified. One yeast isolate showed the highest dye decolorization (96%) after 72 hrs of incubation. The primers (ITS3F and ITS4R) amplified a 380 bp fragment of the

18S ribosomal RNA gene from the most active yeast isolate (Fig. 1). After purification and sequencing, the BLAST-n results showed that the highest score (97%) matches the accession number KF747750.1 in *Saccharomyces. Spp* YC9.13 and (94%) in *Saccharomyces cerevisiae* to the accession number AM262824.1.

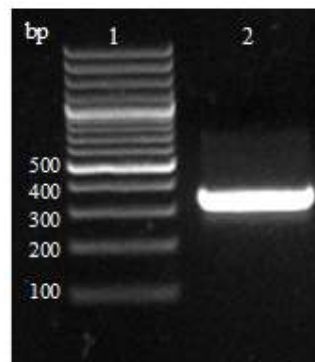


Fig. 1. PCR amplification of the 18S ribosomal RNA gene from *S. cerevisiae* isolate using specific ITS rDNA primers. PCR product was resolved on 1% agarose gel and stained using ethidium bromide. Lane 1, 100bp DNA ladder (Ferments). Lane 2, 380bp ITS rDNA region from our yeast isolate

3.2 Characterization of Nano Zero-valent Irons (NZVI)

3.2.1 X-ray diffraction (XRD)

Fig. 2 shows the powder XRD pattern of NZVI samples under ambient conditions. The broad peak reveals the existence of an amorphous phase of iron. The characteristic broad peak at 2θ of 45° indicates that the zero valent iron is predominantly present in the sample.

3.2.2 Transmission electron microscopy (TEM)

Transmission Electron Microscopy image of iron nanoparticles is shown in Fig. 3. It can be observed that the iron particles are in the form of nano-rods, which exist in contact with each other and form chains having diameters of 5-20 nm. This linear orientation is nearly due to the magnetic properties of iron species [12].

3.2.3 Textural characteristics

The BET surface area values were determined as 34.75 m²/g for nZVI as shown in Fig. 4. Some of the BET surface area values reported in literature are 14.5 m²/g (24), 33.5 m²/g [13] and

36.5 m²/g [6]. The nitrogen physisorption data of nano iron particles showed that the surface area is 34.75 m²/g; total pore volume: 0.09603 cc/g and the average pore radius: 18.596 Å (1.85 nm). Therefore, it is evident that nano-scaled zero-valent irons (NZVI) with good properties were successfully synthesized.

Previous studies [14,15] have shown that Fe⁰ can oxidize organic compounds in presence of oxygen. In this situation, dissolved oxygen is the preferred iron oxidant, resulting in rapid corrosion

according to equation 1. Thus the first step in the oxidation of dye involves the oxidation of Fe⁰ by O₂ (corrosion reaction). This reaction forms a reactive oxygen species either on the particle surface or in solution [16]. H₂O₂ produced in equation 1 could oxidize another Fe⁰ (equation 2) or react with Fe²⁺ (equation 3). The latter is one of most studied advanced oxidation processes, the Fenton's process, which has been widely employed for the efficient degradation and mineralization of a broad range of organic pollutant compounds [17,18].

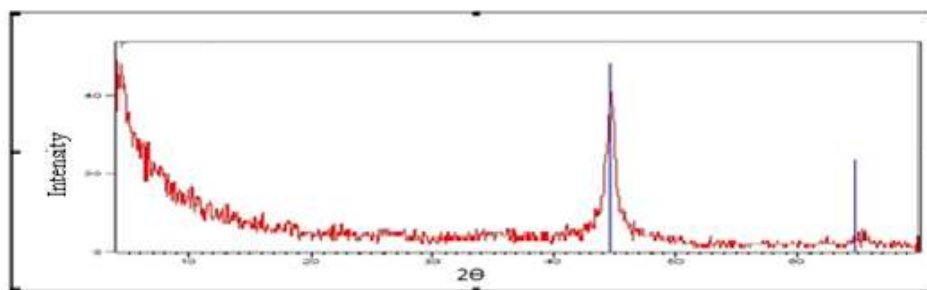


Fig. 2. XRD pattern of zero-valent nanoparticles with weight content of ≈ 90% and Fe₃O₄ as a by-product

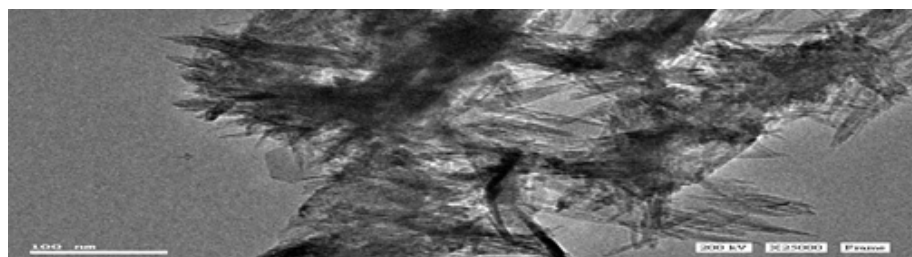


Fig. 3. TEM of zero-valent iron nanoparticles

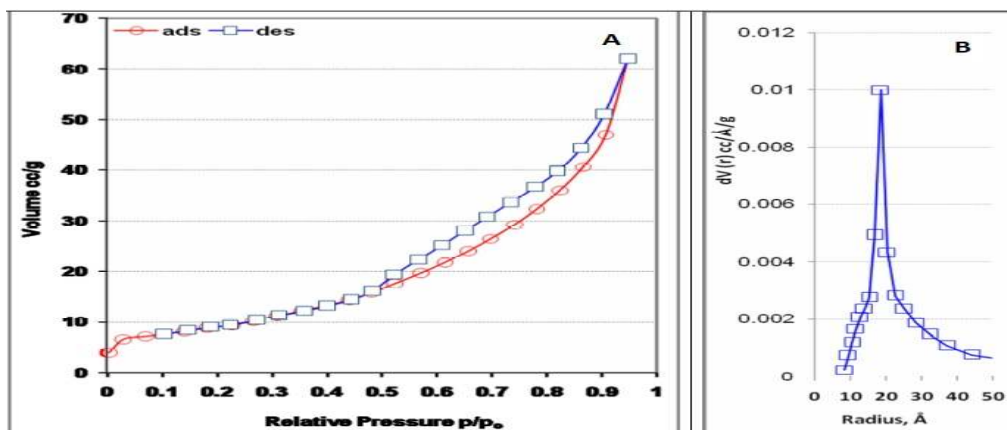
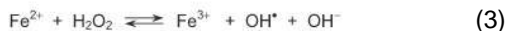
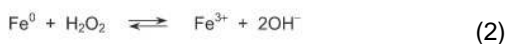
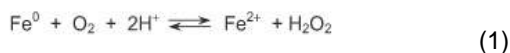


Fig. 4. A) BET adsorption-desorption isotherms of Fe(0) nanoparticles, B) Pore size distribution of Fe(0) nanoparticles



3.3 Decolorization Experiments

The decolorization measurement by spectrophotometer showed that 96% decolorization of Direct Blue 71 was achieved after 72 hr under shaking condition (150 rpm) at room temperature by *S. cerevisiae*. Yeast cells represent an inexpensive and promising method for removal of azo-dyes from textile dye effluents. *S. cerevisiae* have been investigated for decolorization of carmoisine, Reactive Black 5 dye [19]. Decolorization of reactive Black 5 dye by *Yarrowia lipolytica* was enhanced in presence of glucose (5 g l⁻¹) [20]. This result is in agreement with our present study, where supplementation of MSM with 1% glucose in enhanced the yeast decolorization efficiency.

3.4 Effect of NZVI

Effect of NZVI concentration enhanced the dye destruction by the yeast; where complete removal of dye (200 mg/ L) was obtained after 48 hrs. at room temperature in presence of 1 g⁻¹ of the NZVI. A decrease in decolorization at above 1.5 g NZVI/ L was observed. This effect may be

due to toxicity or inhibition of microbial activities. Moreover, presence of 1 g l⁻¹ of NZVI enhanced the decolorization along the incubation time to achieve the maximum decolorization (100%) after 48 hr compared with 90% in the non-catalytic oxidation (by the yeast only, in absence of NZVI) (Fig. 5). On other hand, 78% decolorization was achieved after two days in the control containing dye, ZVI and MSM without yeast. The ZVI have been described to reduce the azo bond in azo dye decolorization in addition to microelectrolysis, and adsorption and flocculation [21-22]. Furthermore, the decolorization process showed similar results under both catalytic and non-catalytic conditions after 60 hr to 72 hr of incubation (Fig. 5).

3.5 Influence of Different of Physio-chemical Different Parameters on Dye Decolorization Percentage

3.5.1 Effect of static and shaking conditions

Under static culture condition, only 20% dye decolorization was obtained (Fig. 6a). This designates that *S. cerevisiae* was a type of facultative aerobe. As is the case for most of the microbial species, oxygen was favorable to the growth of the yeast, as well as for azo dye degradation and enhances the degradation related enzymes, especially the mono-oxygenases and dioxygenases.

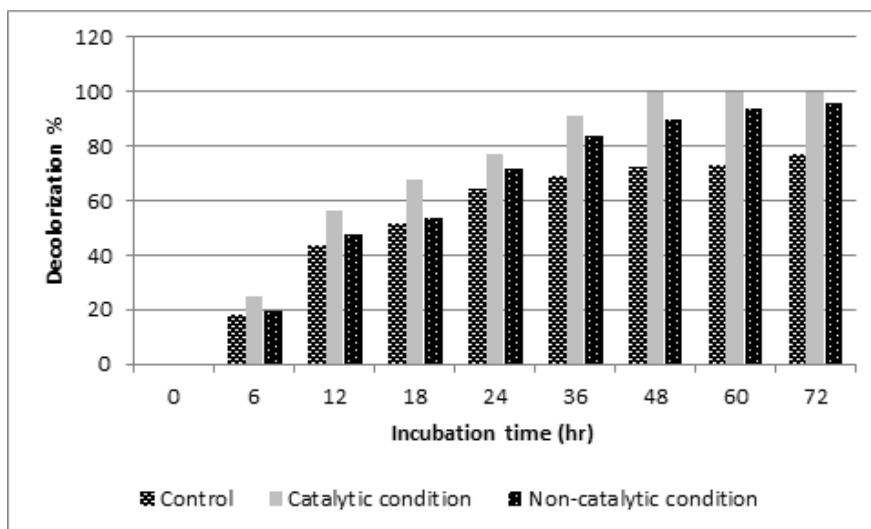


Fig. 5. Decolorization % of Direct blue 71(200 mg/l) by *S. cerevisiae* under catalytic (presence of NZVI 1 g/L) and non-catalytic (absence of NZVI) conditions as compared with the control (MSM with 1 g/L of NZVI)

3.5.2 Effect of pH

Decolorization of Direct Blue71 at various pH values by the *S. cerevisiae* was shown in Fig. 6b. The decolorization percentage was decreased as pH increases. The maximum decolorization was achieved at pH 6.5 (96% and 100%) under non-catalytic and catalytic conditions, respectively. This result was in agreement with Jadhav et al. [23]; where they completely degraded methyl red. at pH range of 5 and 7 by *S. cerevisiae* MTCC 463.

3.5.3 Effect of incubation temperature

The dye decolorization activity was increased with the increase in incubation temperature from 25°C to 37°C with maximum activity attained at 28°C (100%) (Fig. 6c). Further increase in temperature resulted in marginal reduction in decolorization activity of the yeast. Decline in decolorization activity at higher temperature (40°C) can be attributed to the loss of cell viability or to the denaturation of the catalytic enzymes [24].

3.5.4 Effect of initial dye concentration

S. cerevisiae effectively decolorized the dye up to 200 ppm (Fig. 6d); where 100% decolorization was achieved after only 48 hr. The decolorization percentage was decreased at the higher initial dye concentration above 200 ppm. This effect may be due to the toxic effect at these higher concentrations for the yeast. Similar result was obtained [25].

3.6 Identification of Degradation Products

The GC/MS analysis showed presence of twelve major degradation products (Fig. 7). The compounds were identified from its molecular weights and fragmentation pattern guided by the Wiley mass library (Table S1). The degradation products structure suggested that the biodegradation of the Direct-Blue71 azo-dye was catalyzed by activity of four major enzymes: 1) Breakdown of the Diazo bonds by azoreductase, 2) Desulfonation by desulfinase, 3) Breakdown of the aromatic rings by dioxygenases and 4) Oxidation by peroxidases.

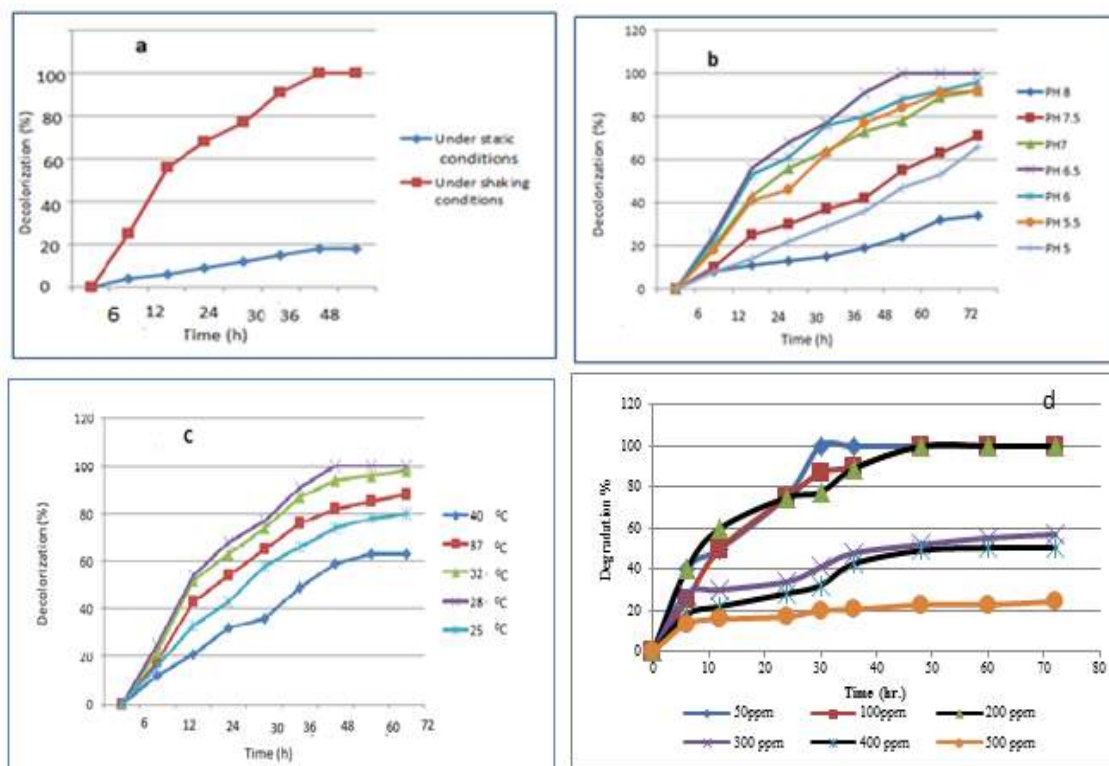


Fig. 6. Effect of shacking conditions (a), pH (b), temperature (c) and dye concentration (d) on decolorization percentage of Direct Blue 71 by *S. cerevisiae*

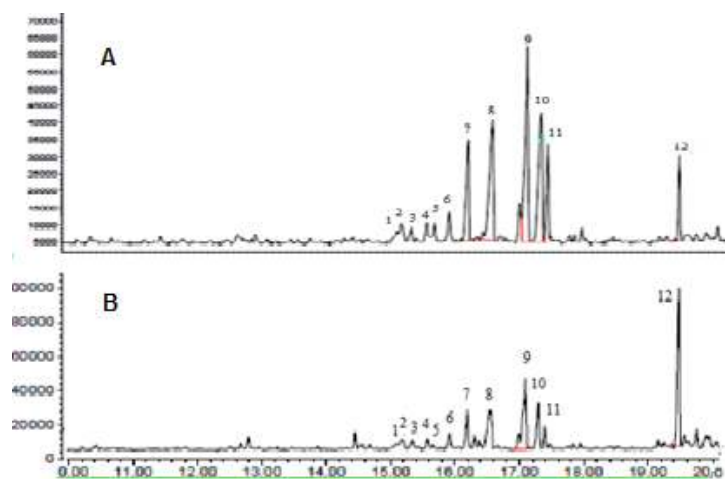


Fig. 7. The GC/MS chromatograms of the *S. cerevisiae* culture extract for the Direct blue azo dye degradation products analysis after 3 days (A) and 5 days (B) of incubation

As showed in Fig. 8, the expected initial step for biodegradation of direct-blue 71 azo dye is the breakdown of the di-azo bonds by azoreductase resulted in sulfonated amino-naphthalene derivatives. This is followed by desulfonation by a desulfinase enzyme. The hydroxyl naphthalene derivative was exposed to subtracting of an electron and H^+ from the hydroxyl phenolic group by peroxidases, generating a phenoxy radical. This radical continue until breaking down the diazo bond and formation of an imine derivative. Presence of 1-hexene,5-imine (Rt. 15.3 min.) in the degradation products reflects the activity of peroxidase [25-27]. Formation of phenoxy radicals followed by sequential formation of carbonium or nitronium ions (compounds 17 to 19, Fig. 8) can be generated by both laccases and peroxidases [26-28]. The bioinformatics analysis in KEGG and Brenda databases confirmed presence of these enzymes in *S. cerevisiae* genome. Their E.C. numbers are: azoreductase E.C. 1.7.1.6; acireductone dioxygenase, E.C. 1.13.11.53 and 1.13.11.54; putative monooxygenase CAT5, E.C. 1.14.13.-; 2-hydroxychromene-2-carboxylate isomerase, E.C. 5.99.1.4 and thioredoxin peroxidase TSA1, E.C.1.11.1.15; while laccases and desulfinase are not detected.

3.7 Enzymes Assay Results

Peroxidase assay showed that the peroxidase production in absence of the dye (in LB medium) was only 0.01 U ml^{-1} , while it was highly increased to 1.07 and 1.02 in MSM supplemented with the dye in presence and absence of NZVI, respectively. Dioxygenase

production in the LB medium was only 0.32 U ml^{-1} ; while it was increased to 0.71 and 0.81 U ml^{-1} in MSM supplemented with the dye in presence and absence of NZVI, respectively. Catalase assay in the LB (control) medium was 0.14 U ml^{-1} , while it was increased to 0.18 and 0.23 U ml^{-1} in the MSM supplemented with the dye in absence and presence of NZVI, respectively. Azoreductase production (U ml^{-1}) in the LB medium was 0.0017; while it was 0.004 and 0.005 in the MSM supplemented with the dye in absence and presence of NZVI, respectively.

The enzymes assay results strongly indicated that presence of the azo dye stimulated the peroxidase, catalase, dioxygenase and azoreductase production; furthermore, presence of NZVI enhanced production of all of these enzymes by *S. cerevisiae*.

Similar investigations have been conducted for biodegradation of azo dyes. The bacterial biodegradation of direct blue 151 and Direct Red 31 (200 mg/L) by moderately alkaliphilic bacterial consortium was investigated [29]. The bacterial consortium decolorized DB 151 and DR 31 by 97.57% and 95.25% respectively, within 5 days. *Staphylococcus lentus* achieved approximately 100% decolorization of Congo red and Eriochrome Black T (100 mg/L) after 24 hr of incubation [30]. *Enterococcus durans* GM13 isolated from textile industry waste water was able to decolorize 87% of Reactive green-19 by 91.3%, Remazol navy blue and Reactive red-198 by 92% within 24 hr of incubation under static condition [31].

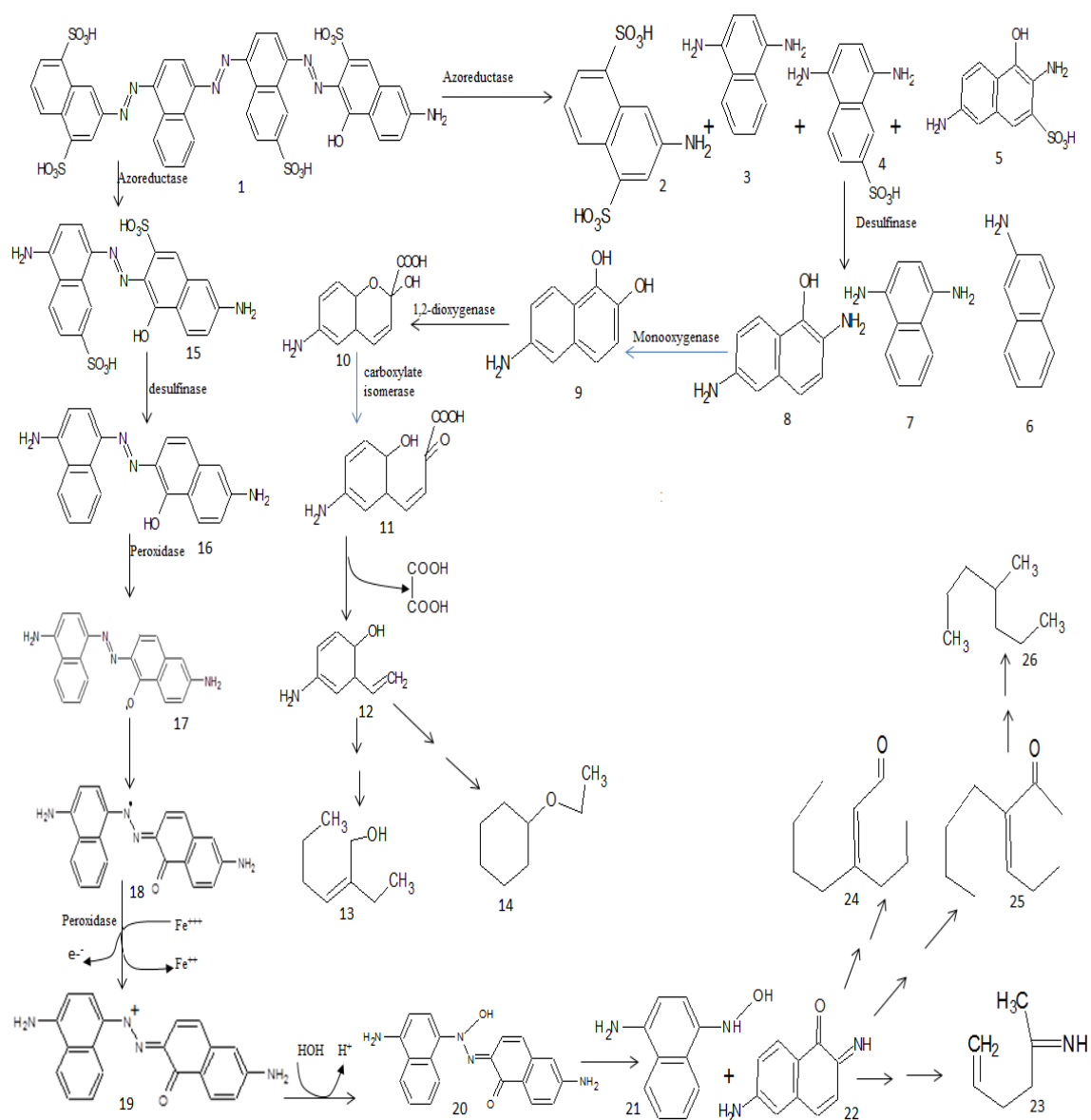
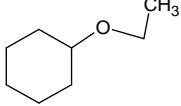
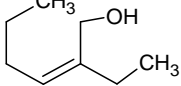
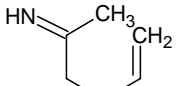
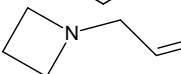
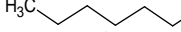
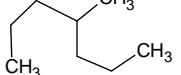
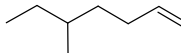
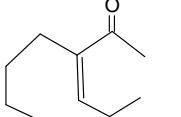
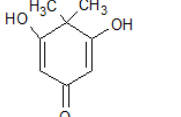
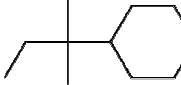
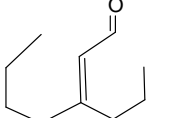


Fig. 8. The proposed biodegradation pathway of Direct-blue azo-dye by *Saccharomyces cerevisiae*. The compounds are: 1) Direct-blue azo-dye; 2) 3-aminonaphthalene-1,5-disulfonic acid; 3) naphthalene-1,4-diamine; 4) 5,8-diaminonaphthalene-2-sulfonic acid; 5) 2,6-diaminonaphthalen-1-ol-3-sulfonic acid; 6) naphthalen-2-amine; 7) naphthalene-1,4-diamine; 8) 2,6-diaminonaphthalen-1-ol; 9) 6-aminonaphthalene-1,2-diol; 10) 6-amino-2-hydroxy-4a,8a-dihydro-2H-chromene-2-carboxylic acid; 11) (3Z)-4-(3-amino-6-hydroxycyclohexa-2,4-dien-1-yl)-2-oxobut-3-enoic acid; 12) 4-amino-6-ethenylcyclohexa-2,4-dien-1-ol; 13) 2-ethyl,2-hexene, 1-ol; 14) Ethoxy-cyclohexane; 15) 7-amino-3-[(E)-(4-aminonaphthalen-1-yl)diazenyl]-4-hydroxynaphthalene-2-sulfonic acid; 16) 6-amino-2-[(E)-(4-aminonaphthalen-1-yl)diazenyl]naphthalen-1-ol; 17) 6-amino-2-[(E)-(4-aminonaphthalen-1-yl)diazenyl]naphthalen-1-ol phenoxy radical; 18) (2E)-6-amino-2-[2-(4-aminonaphthalen-1-yl)hydrazinylidene]naphthalen-1(2H)-one nitronium radical; 19) (2E)-6-amino-2-[2-(4-aminonaphthalen-1-yl)hydrazinylidene]naphthalen-1(2H)-one nitronium ion; 20) (2E)-6-amino-2-[2-(4-aminonaphthalen-1-yl)-2-hydroxyhydrazinylidene]naphthalen-1(2H)-one, 21) N-hydroxynaphthalene-1,4-diamine; 22) 6-amino-2-iminonaphthalen-1(2H)-one; 23) 1-hexene,5-imine; 24) 3-propyl-2-heptenal; 25) 2-heptanone-3-propylidene; 26) 4-methyl, Heptane

Table S1. The biodegradation products of direct-blue71 azo-dye by *Saccharomyces cerevisiae* as appeared from the GC/MS analysis

Ser.	Rt.	Compound name	Structure	Fragments	MW
1	15.1	Ethoxy-cyclohexane		57, 85, 99, 113, 128	128
2	15.2	2-ethyl,2-hexene, 1-ol		56, 69, 81, 85,99, 113, 128	128
3	15.34	1-hexene,5-imine		55, 69, 83, 97	97
4	15.6	Allylazetidine		70, 77, 81, 85, 97	97
5	15.7	Unidecane		57, 71, 85, 99, 113, 156	156
6	15.9	4-methyl, Heptane		58, 70, 85, 98, 114	114
7	16.2	4-decene,8-methyl		70,97,110,125, 138,154	154
8	16.6	2,heptanone-3,propylidene		70, 110, 126, 154	154
9	17.1	2,5-Cyclohexadien-1-one, 3,5-dihydroxy-4,4-dimethyl		70,86,96,112,125,139, 154	154
10	17.3	1,1-dimethylpropyl-Cyclohexane		70,91,110,125,154	154
11	17.4	3,propyl-2,heptenal		70, 97, 110, 154	154
12	19.4	Unknown		75,100, 115,127, 154, 178, 205	205

4. CONCLUSION

Biological processes could be more economical and effective methods for the treatment of colored effluents containing azo dyes that represent a major group of dyes having an environmental concern. In the present study a yeast strain of *Saccharomyces cerevisiae* was isolated from contaminated soil by enrichment culture technique showed extensive decolorization efficiency (100%) of direct Blue71 azo dye in the presence of 1 g l⁻¹ NZVI catalyst after only 48h. The bioremediation of textile dyes and subsequent application of the ZVI have been

largely focused during the last years. This study provides a hope for application of this process in biodegradation of other azo-dyes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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