# Biodegradation of congo red and DDT by *Candida parapsilosis*

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***Abstract***

*Synthetic dyes from textile industrial effluents and DDT (most popular insecticide) have leached into the environment and caused a lot of disturbance in the ecosystem ultimately affecting human lives. Present methods to remediate such pollutants from the environment are not effective enough and rather harming the environment more. Hence, there is a need for effective eco-friendly methods to overcome such problems. Bioremediation is one of the emerging and most favourable techniques to eradicate pollutants from the environment. The purpose of this research was to investigate the bioremediation potential of Candida parapsilosis against synthetic azo dye and the most popular insecticide DDT. Decolorization of congo red dye was determined by UV-Visible spectroscopy. C. parapsilosis was able to remove color substances with decolorization efficiency of 90% when 10 mg/L of dye was used.*

*Furthermore, degradation of DDT was analyzed using High-performance liquid chromatography (HPLC). Significant changes in peak positions were observed as compared to the control spectrum from chromatograms of HPLC which clearly reveal the degradation of DDT. Current work reports the potential of C. parapsilosis* to *bioremediate harmful chemicals of the environment.*

**Keywords:** *Candida parapsilosis*, Congo red dye, DDT, Decolorization, Degradation.

## Introduction

Environmental pollution has become one of the most severe and critical issues worldwide due to increased anthropogenic activities such as urbanization, technological development, unsustainable agricultural practices and industrialization[1]. These human activities have released a myriad of anthropogenic chemicals in water, soil and air. These pollutants have adverse effects on plants, animals, marine ecosystem, soil microbes and soil fertility. This led to a disturbance in the whole ecosystem ultimately affecting human health[2]. Industries release about 10 million tons of hazardous chemicals into the atmosphere per annum globally. Among them, 2 million tons of toxics are known as carcinogens as reported by occupational safety and health administration (OSHA)[3].

In 2018, a total of 3.80 billion pounds of chemicals were released into the air, discharged to water, or improperly disposed of on land[4].The U.S Environmental Protection Agency (EPA) has targeted several classes of chemicals as priority pollutants because of their adverse impact on the atmosphere and human health[5]. Many of these chemicals such as polycyclic aromatic hydrocarbon (PAHs), synthetic azo dyes, pesticides, polychlorinated biphenyls (PCBs), heavy metals and phenolic compounds are considered to be major environmental pollutants due to their carcinogenic effects[6].

Textile dyeing industries use more than 10000 recalcitrant dyes in their process constituting them as a major source of environmental pollution[7]. It is estimated that approximately 280,000 tons of textile dyes were released worldwide into the aquatic ecosystem in 1980s[8].Various dyes are used in the textile industries, of which azo dyes are amongst the most widely used category of dyes (~70%) due to their easy synthesis process, cost-effectiveness, versatility and chemical stability[9]. They are aromatic organic compounds composed of one or more azo groups (-N = N-) which act as a chromophore[10].

Figure 1 shows the structure of the Congo red dye. However, the discharge of azo dyes into the atmosphere has adversely affected all organisms because of their mutagenic or carcinogenic properties[11]. Raj et al[12] reported that azo dyes are toxic, mutagenic, carcinogenic and directly related to human bladder cancer. Biswas and Khuda-Bukhsh[13] showed the cytotoxic and genotoxic effects of an azo dye, through chromosome aberrations and micronuclei test. Studies showed the mutagenic effect of azo dye which leads to the induction of micronuclei in erythrocytes of fishes[14].

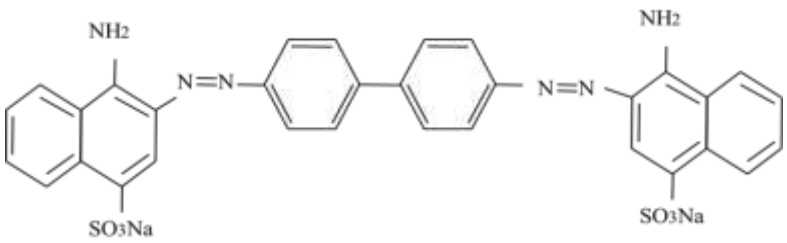


**Figure 1: Structure of Congo red dye**

Similarly, another most common persistent organic pollutant which has affected the ecosystem is dichloro-diphenyl-trichloroethane (DDT) (Figure 2). DDT was the first synthetic pesticide to achieve worldwide attention. It was commonly utilized as an insecticide in agricultural fields and households to control vector-borne diseases and is still used as an agricultural and household pesticide in some countries. However, most of the countries have banned its usage because of its detrimental effects on the environment and human health. Residues of DDT and its metabolites, DDD (1, 1-dichloro-2, 2-bis (4-chlorophenyl) ethylene) and DDE (1, 1-dichloro-2, 2 bis (4 chlorophenyl) ethylene), have been considered as human carcinogens by the United States Environmental Protection Agency (EPA)[15,16].

Various researches have demonstrated that DDT exposure is associated with multiple forms of cancer such as brain cancer, pancreatic cancer, breast cancer and prostate cancer[17-19]. DDT enhances the growth of breast tumor by inhibiting the intercellular communications in human breast epithelial cells[20].

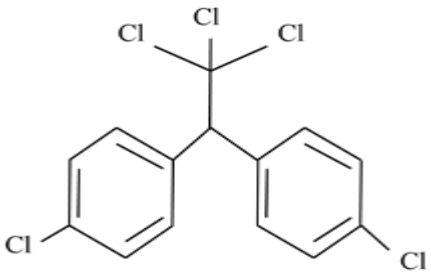


**Figure 2: Structure of DDT**

Eradication of these pollutants from the environment is the foremost requirement of our society in order to reduce their detrimental effects. Existing physical and chemical methods have many drawbacks such as expensive, low efficiency, hazardous by-product formation and high energy requirements[21]. Bioremediation is one of the most promising, cost-effective and eco-sustainable approach to overcome these drawbacks[22]. It is a method of treatment which uses living organisms, primarily microorganism to reduce the toxicity of pollutants[23].

Bioremediation of pollutants can be achieved by two methods: bioaugmentation (addition of microorganism to degrade pollutants) and biostimulation (addition of nutrients, electron acceptor and oxygen to stimulate)[24]. Microorganisms such as fungi, bacteria, algae and yeasts are used in the process of bioremediation[25]. Yeasts have tremendous ability to eliminate contaminants because of their attractive features such as easy availability and can be grown in low-cost growth media[26,27].

Various studies have reported the bioremediation potential of *Candida* species. For instance, *Candida rugopelliculosa* was studied for the removal of the azo dye Reactive Blue 13. The result revealed that *C. rugopelliculosa* could degrade 90% of the dye in 48h[28]. Alcantara et al[29] reported that *Candida cylindracea* has ability to degrade 90% of azo dye (Orange G) after 24h of incubation. Similarly, other species of *Candida* such as *Candida krusei*, *Candida albicans*, *Candida utilis and Candida tropicalis* have also shown bioremediation potential against various dyes.[27,30-33] Furthermore, yeasts also have potential to degrade DDT.

Isia et al[34] investigated the degradation of DDT by a yeast strain *Pichia kluyveri* FM012. According to their results, *P. kluyveri* showed a high degradation rate of 92% within 15 days of incubation. Purnomo et al[35] observed that the brown-rot fungus (BRF) such as *Fomitopsis pinicola* and *Daedalea dickinsii* could effectively degrade DDT having degradation rate of 63% and 47%, respectively[35]. *Alcaligenes sp.* KK have also shown the capability to degrade DDT (66.5% degradation rate)[36].

In our present study, we have studied the remediation potential of the yeast strain *Candida parapsilosis*. *C. parapsilosis* was previously isolated in our lab from a contaminated dairy product[37]. Biosurfactant isolated from the strain had shown antibacterial and anticancer activity[38]. *C. parapsilosis* has also shown resistance against various heavy metals such as Ni, Zn, Cu, Cr, Pb, Hg which confirm the bioremediation potential of the yeast sp.[39]. Here, the bioremediation potential of *C. parapsilosis* was further explored against the most common pollutants, azo dye and DDT.

## Material and Methods

**Chemicals:** For the present study, congo red dye [sodium 3, 3-(1E, 1E)-biphenyl-4, 4-diylbis (diazene-2, 1-diyl) bis (4-aminonaphthalene-1-sulfonate)] was used as synthetic azo dye. It was procured from HiMedia, Mumbai, India. DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane] was purchased from Indo Swiss Chemicals Limited, Chandigarh, India. Pyrene, acetone, n-hexane, methanol, N, N-dimethylformamide (DMF) and potato dextrose broth (PDB) were obtained from Hi Media, Mumbai, India.

**Microorganism and Culture Conditions:** Previously isolated *C. Parapsilosis* was grown on PDB media and incubated at 37°C for 48 hours as reported by Garg et al[38].

**Stock solution preparation:** Congo red1 g of dye was dissolved in 100ml of distilled water to obtain 10 g/L stock solution of dye. It was further filter sterilized by using 0.2 µm syringe filters.

**DDT**: 5 mM of working solution of DDT was prepared in DMF. Pyrene was taken as an internal standard and its 5 mM working solution was prepared in DMF.

**Dye Decolorization experiments at different dye concentrations:** The decolorization of congo red was demonstrated at different concentrations (10, 100, 200, 500 mg/l) of dye in 250 ml Erlenmeyer Flasks. Uninoculated

medium without dye was set as a blank and uninoculated medium with dye was used as control. The stock solution of the dye was separately prepared and filter sterilized. It was aseptically added to 100 mL PDB medium to a final concentration of 10, 100, 200 and 500 mg/L in different flasks. The flasks were inoculated with a freshly grown cell suspension of *C. parapsilosis* and incubated under shaking conditions (150 rpm, 37°C for 4 days).

Visual screening of dye decolorization was observed before UV-Vis spectrometry analysis after the incubation period. Then, aliquots (2ml) of the culture media were withdrawn from the flasks for color measurement. Suspended particles were separated by centrifugation at 6000 rpm for 10 min. Similarly, aliquots (2ml) were also withdrawn from the control flask and blank. Abiotic controls (without microorganism) were always included. All experiments were carried out in triplicate and mean values were taken[31,40].

**Decolorization Assay:** Decolorization was examined by analyzing the absorbance of the respective cell-free supernatants at the absorbance maxima of the dye (498 nm) using a UV-VIS-Spectrophotometer (Perkin Elmer). The efficiency of decolorization was determined according to the following equation:[40]

Decolorization (%) = [(Initial Absorbance – Observed Absorbance) / Initial Absorbance] × 100

where initial absorbance is the absorbance of the uninoculated medium with dye (Control) and observed absorbance is the dye absorbance after decolorization by the culture.

**Biodegradation of DDT by biosurfactant producing *Candida parapsilosis*:** For determining the biodegradation of DDT, the culture of *C. parapsilosis* in PDB medium was statically pre-incubated at 37°C for 3 days. After the pre-incubation period, 50 µL of 5 mM DDT solution in DMF (final concentration, 6.25 µM) was added to each flask inoculated with culture. The flasks were then sealed with paraffin tape to avoid the volatilization of the substrate. They were incubated for 7 days at room temperature at static conditions. The cultures were killed by autoclaving at121°C for 20 minutes after pre-incubation for control[12,41,42]. The experiment was performed in triplicate.

**HPLC analysis of DDT and its metabolites:** HPLC analysis of DDT and its metabolites was performed by using a system equipped with Luna 5u c18 100A 250 x 6.4 mm 5-micron column. Pyrene was taken as an internal standard. The same concentration of pyrenein DMF as DDT (0.25 µmol) was added to each sample. The samples were mixed with 40 mL of methanol and washing was done with 120 mL of acetone. Then, they were filtered via a syringe filter and the filtrates were mixed and evaporated. The organic fraction was further collected by extracting the filtrate with 250 mL of n-hexane. The extracts were evaporated at 64°C and dried under low pressure. The obtained concentrate was re-dissolved in methanol. Its analysis was done by using high-performance liquid chromatography (HPLC) (Waters Micromass QT of Micro). The samples were eluted with 82% methanol in 0.1% trifluoroacetic acid aqueous solution at a flow-rate of 1 mL/min for 15 min at 254 nm. Retention time and absorption maximum at specific wavelengths of DDT and its metabolites were analyzed and compared with authentic standards[43].

## Results and Discussion

**Visual Screening of Decolorization of Congo red dye:** After the incubation period of *C. parapsilosis* with congo red dye, color of the culture medium and yeast cells was observed. Although, these observations are not enough to predict the precise decolorization efficiency, but still they give an idea about the decolorization ability of the yeast strain. As shown in figure 3, it is clear that the yeast cells have adsorbed the red color of the dye. The cell pellet of the culture is red and the supernatant is clear (Figure 3 B) (the yellowish color is due to PDB media) which shows that decolorization has taken place. The possible mechanism involved is biosorption of the dye on yeast cells as mentioned in most of the studies[28,33,44].


**Figure 3: Visual Screening of decolorization of congo red (10 mg/L): A: From left to right- PDB media (Blank), PDB media with dye (Control) and inoculated culture of *C. parapsilosis* with congo red dye incubated for 4 days at 37°C; B: Aliquot of dye-containing culture media after centrifugation.**

**Decolorization efficiency of Congo red dye by *C. parapsilosis*:** The decolorization efficiency of Congo red by *C. parapsilosis* was assessed by UV-Vis spectrophotometry. The strain was tested against different concentrations of dye for 4 days (Figure 4). Shaking conditions were maintained throughout the experiment as shaking favors the decolorization of dye as compared to static conditions[45]. *C. parapsilosis* could decolorize dye well at concentrations 10 mg/L and 100 mg/L (90% and 86% respectively) under the given conditions (reaction time four days, 150 rpm, 37°C and pH 7.0). As the concentration of dye increased, decolorization was reduced, may be because of the toxicity of higher concentration of dye for cell growth[41].

Previous studies were similar with these findings. Decolorization efficiency decreased with the increase of dye concentration[32,41,46]. Dye concentration in most of the industrial effluents ranged from 10-250 mg/L as mentioned by Ghaly et al[47]. Sivakumar[48] reported that 45 mg/L of the dye Acid Orange 10 is discharged from the final clarifier of a textile factory in India.

Thus, dye concentration >200mg/L is high enough to be equivalent to that of industrial dye wastewater[33]. Yu et al[33] reported that *Candida palmioleophila* was able to achieve 98% decolorization rate with the azo dye Reactive Brilliant Red K-2BP after 24 h. In another study, *C. palmioleophila* was able to decolorize up to 85.7% of the Reactive Black 5 (200 mg/L) after 24h at 35°C.

Decolorization efficiencies for other azo dyes by this strain were 53.35-97.9% after 24 h[31]. *Candida albicans* has been reported to degrade 73.2% Direct Violet 51 azo dye of concentration 100µg/ml[27]. The decolorization rate of Remazol Turquoise Blue-G reactive dye (50 mg/L) by *Candida utilis* was observed to be 82%[30].

Tan et al[32] studied the decolorization of Acid Brilliant Scarlet GR by *Candida tropicalis* TL-F1. According to their results, *C. tropicalis* showed 97.2 % of decolorization rate when dye concentration was 20 mg/L after 19 h of incubation. However, at 500 mg/L concentration of dye, 58.8 % degradation efficiency was observed[32]. Studies on Congo red dye also showed similar results. Bhattacharya et al[45] studied bioremediation of congo red dye (0.005% or 50 mg/L) by various fungal species. Among six fungal strains, only *Aspergillus flavus* showed best decolorization rate (98.86%) after 4 days. However, other fungal species had decolorization rate ranging from 10-60% with similar culture conditions. These observations suggested that *C. parapsilosis* might be a potential strain for the remediation of textile industry effluents.

Furthermore, the literature suggests that heteropolysaccharides and lipid components present in the cell wall of yeast cells assist in the biosorption of dyes. These components consist of various charged functional groups such as carboxyl, hydroxyl, phosphate etc. which results in strong attractive bonds between dyes and cell wall[11]. Another mechanism involved in the degradation of dyes, simultaneous with biosorption, is the enzymatic pathway. Peroxidases and laccases are mainly involved in the degradation of dyes[31]. In the future, enzymatic assays could be done to precisely determine the biodegradation level of azo dye by *C. parapsilosis*.

**Degradation of DDT by *C. parapsilosis***: Retention time (RT) and maximum absorption at specific wavelengths were analyzed to determine the presence of DDT and its metabolic by-products. The absorption spectra of the samples and control obtained at 254 nm are presented in figures 5 and 6. The retention time (RT) of the internal standard (pyrene) was observed at 2.779 minutes (Figure 5).

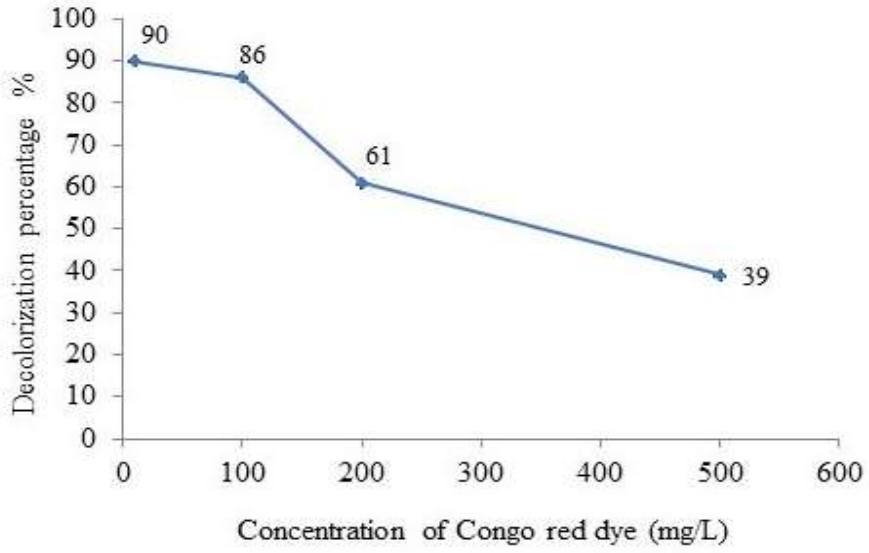


**Figure 4: Decolorization efficiency of Congo red dye by *C. parapsilosis* after incubation for 4 days.**

The elution profile obtained for the *C. parapsilosis* treated samples was significantly different from the control in terms of the number of peaks obtained, the height of peaks and RT. The HPLC profile of DDT treated with *Candida sp.* showed 2 major peaks with RT 4.921 and 5.367 min (Figure 6). On comparing with the peaks that appeared at the beginning of static incubation, it was observed that the corresponding peaks were absent in the treated sample and new peaks were observed (DDD and DDE) which indicate that DDT has been degraded.

Similarly, Mwangi et al[49] had observed the absence of DDT peak and appearance of its two metabolites peaks in the sample treated by bacterial isolates. Thus, the present study confirms that *C. Parapsilosis* has the ability to degrade DDT. The possible reason for DDT degradation is the role of biosurfactant produced by *C. parapsilosis*.

In our previous study, it was confirmed that *C. parapsilosis* produces biosurfactant and has resistance against certain heavy metals[38,39]. A study done on biosurfactant producing *Pseudomonas aeruginosa* has revealed that the biosurfactant producing strain had enhanced the degradation rate of DDT[36]. However, the present study was based on a single time and concentration of DDT. Also, quantitative analysis is to be done in the future to precisely predict the degradation capability of the yeast strain.

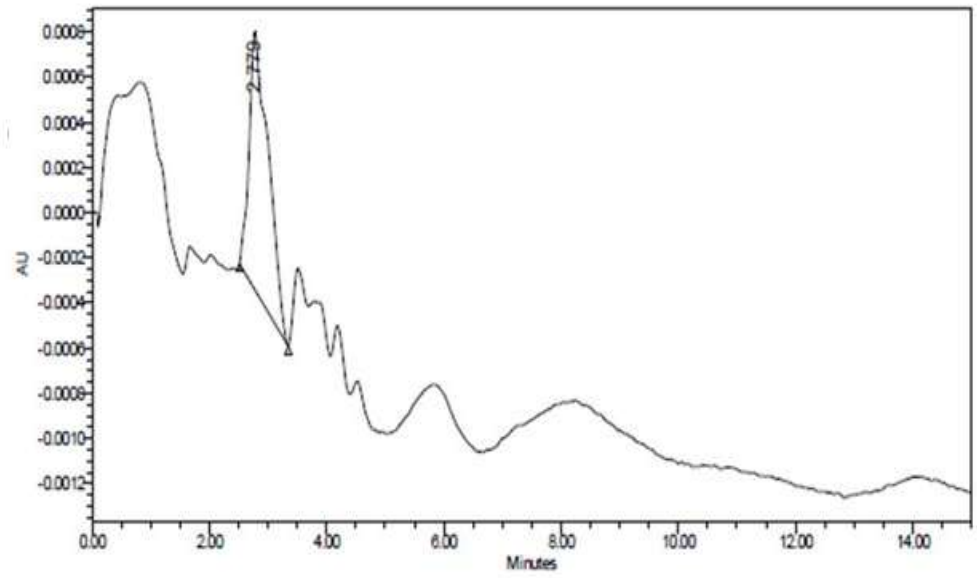


**Figure 5: HPLC Chromatogram of control**

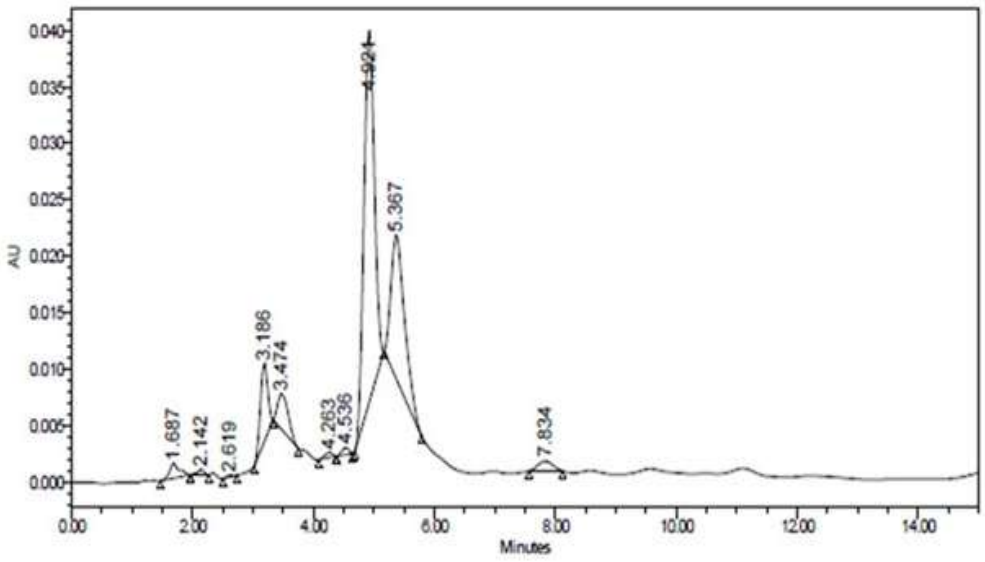


**Figure 6: HPLC Chromatogram of the treated sample**

## Conclusion

This study demonstrated the bioremediation capability of *Candida parapsilosis.* It was evident from the current study that the yeast strain was able to decolorize and degrade the two most common pollutants, azo dye and DDT. The yeast cells could decolorize up to 90% of congo red dye within 4 days and decolorization efficiency would decrease if the concentration of the dye is increased. However, the present study was conducted on a single parameter (concentration) and can be considered as a preliminary report. In future, other parameters such as effect of variation of temperature and pH as well as use of other dyes could also be considered to study the effectiveness of the strain. Additionally, enzymatic assays could be done to confirm the enzymes responsible for the degradation of azo dye by the yeast strain.

Further, the degradation of DDT was observed by HPLC analysis. The chromatograms showed the peaks of DDD and DDE, which are metabolites DDT in the treated sample. Biosurfactants produced by the yeast species could be the reason for DDT degradation. However, this study was preliminary. The quantitative analysis could be done in the future to precisely confirm the degradation efficiency of the yeast strain. Therefore, further research should be undertaken to understand the bioremediation potential of *Candida parapsilosis* in detail.

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