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Biodegradation of Congo Red from Liquid Medium using Fungi Isolated from Dye Contaminated Sites

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Abstract—*Azo dyes are released into wastewater streams without any pretreatment and pollute water and soil environments. To prevent contamination of our vulnerable resources, removal of these toxic dyes is of great importance. Dye decolourization with microorganisms is low cost effective and eco-friendly and the only means for ultimate controlling of pollution generated by textile industries. In the present study, an attempt was made to examine the potential of fungal isolates for decolourization of congo red textile dye from liquid medium. Decolourization of azo dye congo red by two isolated fungal species, Aspergillus flavus and Aspergilus niger has been analyzed using potato dextrose broth (PDB) medium containing 100ppm of congo red at different physico-chemical parameters like, pH, incubation time, inoculum volume, dye concentration and carbon sources. Aspergillus flavus was able to decolorize the congo red at pH 4, 6% inoculums, 1% glucose and 100ppm dye concentration whereas A. niger was able to decolorize the congo red at pH 4, 3% inoculums, 1% glucose and 200ppm dye concentration. Extent of decolourization recorded by A. flavus was 95% and that by A. niger was 97%. The study has confirmed the potential of the above fungi in the decolourization of the textile dye congo red efficiently and opened scope for future analysis of their performance in the treatment of textile industry effluent. However, more and more research works are desired to develop a feasible alternative process for the treatment of textile effluents.*

Keywords*: Azo dyes, Congo red, Decolourization, Microorganisms, Pollution, Textile effluent*

1. INTRODUCTION

Water is a limited, valuable and predetermined resource which is incessantly under threat due to a range of various human activities, particularly arising from inappropriate discharge of industrial effluents. The textile industry is one of them, which widely use synthetic chemicals as dyes. Wastewaters from textile industries pose a severe hazard to the environment, as large amount of chemically diverse dyes are used. Wastewater from the textile as well as other dye-stuff industries contains substantial amounts of synthetic dyes that require treatment to prevent ground-water contamination. Along with increasing textile industries, the environmental pollutants like the synthetic complex organic dyes as the coloring substances are also spreading all over the world. The textile industry covers two-third of the gross dye stuff market. The yearly world production of textiles is about 30 million tones including 700,000 tones of different dyes [25] which cause severe environmental pollution problems. During manufacturing and usage, approximately about 10- 15% of the dye is lost directly to water bodies that find its way into the environment [6, 7]. During recent years, water pollution due to textile industry effluent has increased. In addition, it is very hard to treat textile industry effluents, because of their high chemical oxygen demand (COD), biological oxidation demand (BOD), color, pH and the presence of metal ions [1]. Color is an imperative property of human appearance and gives pleasant pleasure to eyesight but at the same time they may act as severe pollutants when their origin is dyes and dyestuffs. In 1856, William Henry Perkin revealed the world's first commercially successful synthetic dye. Color present in the industrial effluent gives a clear-cut indication of water being infected. Hence color is the first noxious waste to be recognized in the textile effluent and has to be removed prior to discharging into water bodies or on land [10].

Azo dye constitutes the principal class of synthetic dyes used in a number of industries such as textile, dyeing, cosmetics, paper, printing, food, among the textile industry as the leading consumer. Azo dyes have been used increasingly in industries because of their cost effectiveness and no difficulty in synthesis as compared to natural dyes. Textile industry is famous for using huge quantities of water and diversity of chemicals [21]. Azo dyes are characterized by reactive groups that form covalent bonds with OH─, NH─, or SH─ groups in fibres (cotton, wool, silk, nylon). Almost one million tons of azo dyes are annually produced in the world, of which azo dyes characterized by an azo bond (R_1-N=N-R_2), represent about 70% by weight [8]. Azo bonds present in these compounds are resistant to breakdown, with the potential for the perseverance and accumulation in the surroundings [14]. Azo dyes constitute a major class of environmental pollutants and the discharge of these brightly colored industrial effluents adversely affect water bodies, soil fertility, aquatic organisms and ecosystem integrity. Dyes make the world more delightful through

coloured substances, but on the other hand they represent a serious threat for the environment. The discharge of azo dyes in water bodies is challenging not only for aesthetic reasons, but also because the azo linkage of azo dyes may undergo metabolic cleavage resulting in free aromatic amines which are renowned as possible human carcinogens [2]. So azo dyes contaminated effluents because of their expulsion into the environment is of great concern in present days due to colouration of natural waters and also due to toxicity and these Azo dyes are xenobiotic in nature and in some cases are mutagenic and carcinogenic [4, 5].

Various physiochemical methods can be used for the removal of azo dyes from waste water such as adsorption, ultra-filtration, ion-exchange, chemical oxidation, electrolysis etc. [22]. However, all these physiochemical methods are quite expensive and result in the production of huge amounts of sludge, which creates the secondary level of land pollution [12, 13]. Therefore, in such situation, biological treatment has been considered as alternative remediation for decolorizing textile azo dyes [8, 18]. Bioremediation through microorganisms has been identified as a cost effective and eco-friendly alternative for removal of textile effluent [3, 19]. Bioremediation is the most desirable approach for cleaning up the toxic environmental pollutants. Bioremediation is a pollution control technology that uses natural biological species to catalyze the degradation of a variety of toxic chemicals to less harmful forms [24] and in many cases, adsorption of dyes to the microbial cell surface is the primary mechanism for decolourization [23]. Microorganisms like bacteria and fungi have showed their ability to degrade and bio-transform azo dyes from wastewater [2].

This study aims to investigate the potential of fungi isolated from dye industry effluents for decolourization of a textile dye, congo red and effect of various process parameters like pH, incubation time, inoculums volume, dye concentration, carbon source.

2. MATERIAL AND METHODS

2.1. Chemicals and media

All chemicals used in this study were of AR grade. Textile dye, congo red was collected from a dye Industry. The textile industry effluent samples were collected from dye-contaminated sites of industries in Haryana and Chandigarh. Media and carbon sources used were purchased from Hi-Media Laboratories (Mumbai, India).

2.2. Isolation, screening and identification of dye degrading fungi

Collected samples were used for isolation of dye decolorizing fungal cultures by enrichment culture techniques. One ml of effluent was transferred into 9 ml of distilled water in sterile test- tubes. This stock solution was by serially diluted to get concentration ranging from 0.1 ml of sample from each dilution was spread on potato dextrose agar (PDA) plates with the help of L-rod. The petriplates were incubated at 30^{o}C for 5 days. A plug of mycelium of the fungal isolate was placed on a clean slide containing a drop of lacto phenol cotton blue (LCB) solution. The mycelium was spread using a sterile needle and a clean cover slip was placed above the preparation and observed under the light microscope for the identification of fungal isolate.

2.3. Preservation and maintenance

Pure fungal isolates were obtained on the PDA plates by sub-culturing. The isolates were further sub-cultured on PDA slants and incubated at 30^{o}C. After sufficient growth of the colonies, the slants were stored in refrigerator and served as stock cultures. Every 30 to 60 days sub-culturing were routinely done.

2.4. Spore suspension preparation

A mycelium disc of 1.2 cm diameter obtained from a 5 days old culture plates of fungus were transferred to 100ml PDB in a 250 ml conical flask and incubated at 30^{o}C for 5 days and the flasks were kept in a shaker. Then the content of each conical flasks were filtered through filter paper.

2.5. Screening of decolourizing fungi

All the isolates were selected for screening of decolorizing activity of the dye congo red. Inoculums (3%) of each isolate were added to 100 ml of Potato dextrose broth (PDB) supplemented with 100ppm dye effluent and incubated at 30°C for 5-6 days. After 5-6 days, efficient decolourization was seen visually. Those isolates showing decolourization of textile dye effluent were selected for further studies on optimization of physico-chemical parameters.

2.6. Decolourization assay

Dye Decolourization activity was expressed in terms of percentage decolourization and was determined by monitoring the decrease in absorbance [15]. Fungal culture with dye in PD broth was filtered for removal of biomass. The degree of decolourization of the dye was measured at its respective maximum absorbance wavelength (495nm) for congo red using supernatant by UV-visible spectrophotometer (1800, Shimadzu, Japan). The decolourization assay was calculated according to the following formula [15].

$$D= [(A0-A1) /A0] \times 100$$

Where, D=decolourization in %; A0=initial absorbance; A1= final Absorbance

2.7. Optimization of dye decolourization

Decolourization of congo red textile dye (100ppm) in PD broth by two isolates was optimized with respect to the effect of pH (2, 3, 4, 5, 6,7), incubation period (48, 72, 96, 120, 144, 168 hrs), inoculums volume (3%, 6%, 9%, 12%, 15%), dye

concentration (100-500ppm), 1% carbon sources (maltose, glucose, sucrose, lactose, starch).

Influence of the volume of inoculums was evaluated by inoculating 3%, 6%, 9%, 12% and 15% of respective cultures to PDB media containing Congo red. All the flasks were incubated at respective temperature mentioned above under shaking conditions for 5-6 days. The time course of decolourization was monitored under optimum conditions. Flasks were incubated for 5-6 days at their respective temperature and samples were removed after every 24 h and analyzed for decolourization activity as described above.

3. RESULTS AND DISCUSSION

Decolourization of textile dye effluent is severe environmental dilemma, which is clear from the magnitude of research done in this field in the last decade. In recent years, various physiochemical methods have been opted for treatment of dye effluents but the bioremediation using microorganisms have been considered as an alternative and eco-friendly approach for degradation of toxic dyes. Dye decolourization through biological means has gained drive as these are cheap and can be applied to wide range of dyes. In the present study microbial decolourization of textile dye congo red was carried out using the fungal isolates obtained from the textile dye effluent.

3.1. Isolation and identification of fungi from textile dye effluent

Isolation of fungi capable of decolorizing textile dye congo red was carried out on PD broth from textile effluent samples collected from dye contaminated sites. A total of 20 fungal isolates were isolated and screened for their potential to decolorize congo red from liquid medium. Of these, two isolates (FCR3 and FCR9) were selected for further studies based on their ability to degrade congo red from liquid medium efficiently and rapidly showing more than 95% decolourization. The fungi obtained were identified on the basis of morphological, microscopic observations and cultural characteristics (Table-1).

Table 1: Identification of fungal isolates

Colony morphology and microscopic observation	Purified colonies	Isolates
Colonies yellow at first, quickly becoming bright to dark yellow green; conidiophores coarsely roughened up to 1mm long, and 19-20 μm diameter	FCR3	Aspergillus flavus
Colonies spreading rapidly, with mycelium white to dark brown to black or purple conidial heads; conidia small, more or less globose, rough, 4-5 μm diameter	FCR9	Aspergillus niger

The isolates FCR3 and FCR9 were further identified as *Aspergillus flavus and Aspergilus niger* respectively. Identification of these two fungal isolates was done at Department of Plant Pathology, Indian Agricultural Research Institute, PUSA, New Delhi. From the literature survey various fungus such as *Fusarium oxysporum, Altermaria altermata, Chaetomium globosum, Trichoderma viride, Penicillium notatum, Aspergillus flavus, Aspergillus niger* were reported for decolorization and degradation of widely used azo textile dyes [15, 11]

3.2. Effect of pH

Figure–1 illustrates the effect of different pH on decolourization of congo red by *A. flavus* and *A. niger*. From the above data it can be inferred that both fungi *A. flavus* and *A. niger* shows efficient decolourization of congo red at pH 4 recording 96% and 94% decolourization of the dye respectively. Ponraj [20] reported that the percentage of decolourization of true blue by *Aspergillus niger* and *Mucor* sp. was about 97.52% at pH 6 and 84.87% at pH 4.

3.3. Effect of incubation time

The time course of decolourization of congo red by *A. flavus* and *A. niger* was illustrated in Fig. 2. The results have indicated the fact that both *A. flavus and A. niger* are capable of promoting nearly 95% decolourization of congo red during incubation period of 96 hours.

Decolourization of textile dyes by fungi has been investigated extensively, reporting wide range of combinations of dyes and fungi. Current study has confirmed the decolourization efficiency of two fungal strains, *A. niger* and *A. flavus* in decolorizing the textile dye congo red. Comparative analysis of the time course of decolourization by the two fungi under their respective optimal conditions has revealed high order of activity by *A .flavus and A. niger* recording almost 95% of decolourization. Mohammed [16] reported that the percentage of decolourization of congo red dye by *A. flavus and A. terreus was* about 46.89% and 34.59% during incubation period of 72 hours. Therefore, it can be concluded that both the fungi are good means in the decolourization of textile effluent contaminated with dyes.

3.4. Effect of inoculums volume

Influence of the inoculums volume on decolourization of the dye congo red by *A. flavus* and *A. niger* is presented in Fig. 3. From the data it is observed that *A. flavus* and *A. niger* are effective in decolourizing congo red recording maximum decolourization 95% and 96% of the dye. The ideal volume of inoculum was found to be 6% for *A. flavus* and 3% for *A. niger.*

3.5. Effect of dye concentration

In order to study the effect of initial dye concentration of congo red, the experiments were carried out at different dye

concentrations (100ppm, 200ppm, 300ppm, 400ppm and 500ppm). Based on the graph in Fig. 4, the maximum dye decolourization was found to be in the concentration of 100ppm and 200ppm which was 91% and 96% after treated with *A. flavus and A. niger* for 96 hours of incubation respectively. The decolourization of congo red indicated the degradation of azo bond and further treatment by fungi can lead to the cleavage of aromatic rings [9]. Further increase in the dye concentration upto 300pmm- 500ppm, decreased the rate of decolourization. This is due to the increase in the toxicity of dye that inhibited the growth. The higher concentration of azo dye inhibits nucleic acid biosynthesis and cell growth [3], so the effect of dye concentration on growth of organisms is an important consideration for its field application. Besides, azo dye is a recalcitrant and complex molecule for degradation as it consists of fused aromatic rings. Even though this, *A. flavus and A. niger* still have the potential to decolorize high concentration of dye.

3.6. Effect of carbon source

Fig. 5 illustrates the effect of different carbon sources on decolourization of congo red *by A. flavus* and *A. niger*. Glucose has emerged as the ideal carbon source for both the fungal isolates, both recording highest rate of decolourization. Of the two fungal strains tested, *A. niger* exhibited highest decolourization activity recording 97% and by *A. flavus* 95%. Ponraj [20] used fructose as a carbon source for decolourization of true blue using *A. flavus* accounting 94.76% decolourization. Nosheen [17] used glucose and starch as carbon sources for maximum decolourization of Reactive Black B and Reactive Orange 16.

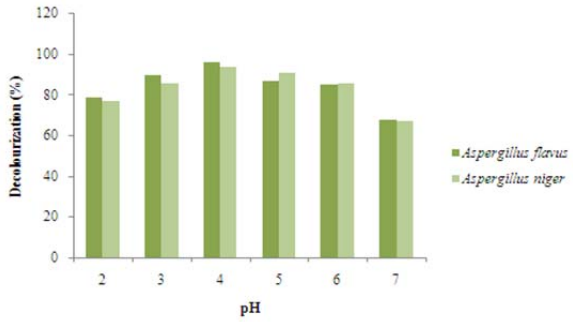

Fig. 1: Effect of pH on decolourization of congo red from liquid medium using fungi

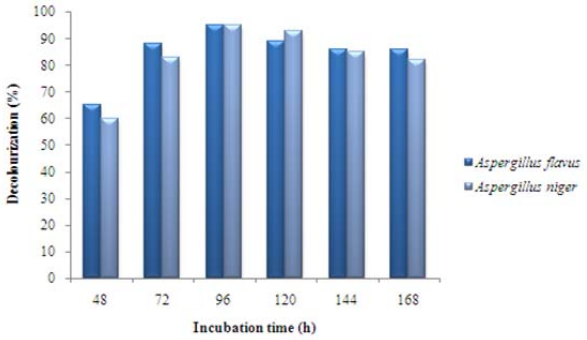

Fig. 2; Effect of incubation time on decolourization of congo red from liquid medium using fungi

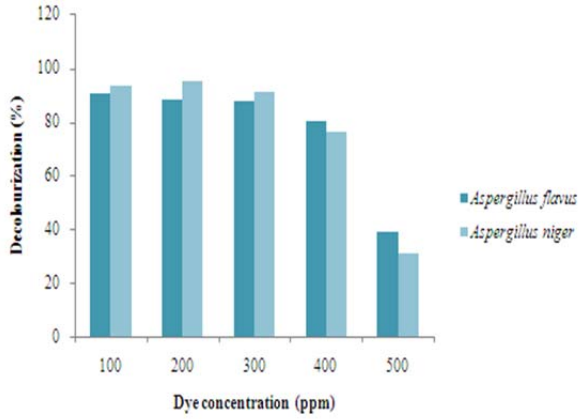

Fig. 3: Effect of inoculum volume (%) on decolourization of congo red from liquid medium using fungi

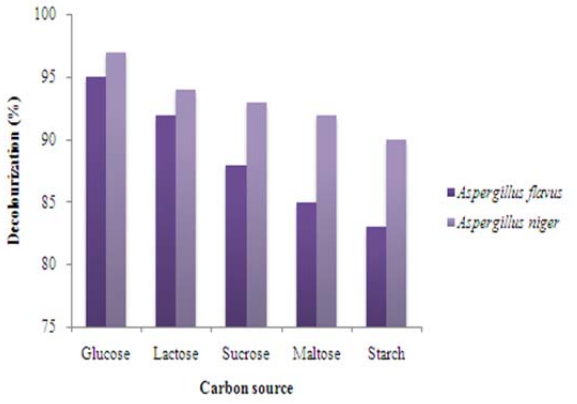

Fig. 4: Effect of dye concentration on decolourization of congo red from liquid medium using fungi

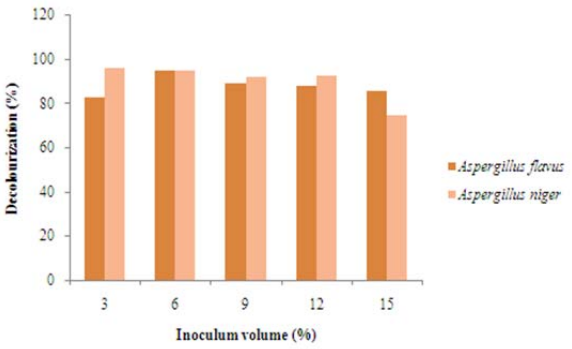

Fig. 5: Effect of different carbon sources on decolourization of congo red from liquid medium using fungi

4. CONCLUSION

In the present study, *Aspergillus flavus* and *Aspergilus niger* were used for decolourization of textile dye congo red. Both thefungi were found to be the most effective in decolourization of azo dye-congo red, resulting in almost complete colour removal at the end of fourth day of incubation period. To conclude, the selected isolates appear to be an attractive alternative for the treatment of industrial effluents contaminated with dyes. *Aspergillus flavus and A. niger* can be used for the treatment of textile effluents and can be performed in low cost at the industrial site.

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REFERENCES

[1] Anjali, P., Poonam, S., and Leela, I., "Bacterial decolorization and degradation of azo dyes", *Int Biodeterior Biodegradation,* 59, 2007, pp. 73-84.

[2] Banat, I. M., Nigam, P., Singh, D., and Marchant, R., "Microbial decolorization of textile-dye containing effluents: a review", *Bioresource Technology*, 58, 1996, pp. 217-227.

[3] Chen, K.C., Wu, J. Y., Liou, D.J., and Hwang, S. C., "Decolorization of the textile dyes by newly isolated bacterial strains" *J Biotechnol*, 101, 2003, pp. 57-68.

[4] Daneshvar, N., Ayazloo, M., Khatae, A. R., and Pourhassan, M., "Biological decolorization of dye solution containing malachite green by microalgae *Cosmarium sp." Bioresource Technology*, 98, 2007, pp. 1176- 1182.

[5] Dafale, N., Agrawal, L., Kapley, A., Meshram, S., Purohit, H., and Wate, S., "Selection of indicator bacteria based on screening of 16S rDNA metagenomic library from a two-stage anoxic–oxic bioreactor system degrading azo dyes", *Bioresource Technology*, 101, 2010, pp. 476–484

[6] Elisangela, F., Andred, Z., Fabiana, F. G., Isis, S. S., Artur, C. P., and Luica, R. D., "Microaerophilic–aerobic sequential decolourization and biodegradation of textile azo dyes by a facultative *Klebsiella sp*." *Process Biochem*, 22, 2009, pp. 576-582.

[7] He, F., Hu, W., and Li, Y., "Biodegradation mechanisms and kinetics of azo dye 4BS by a microbial consortium", *Chemosphere*, 57, 2004, pp. 293-301.

[8] Hao, O. J., Kim, H., and Chang, P. C., "Decolourization of wastewater, critical rev" *Environ. Sci. Techno*l, 30, 2000, pp. 449-505.

[9] Hadibarata, T., Yusoff, A. R. M., Aris, A., Salmiati, Hidayat, T., and Kristanti, R. A., "Decolorization of azo, triphenylmethane and anthraquinone dyes by laccase of a newly isolated *Armillaria sp*. F022", *Water, Air, and Soil Pollution*, 223, 2012a, pp. 1045–1054.

[10] Khadijah, O., Lee, K. K., Mohd, Faiz, F., and Abdullah., "Isolation, screening and development of local bacterial consortia with azo dyes decolourising capability" *Malaysian Journal of Microbiology*, 5, 1, 2009, pp. 25-32.

[11] Laxmi1, S., and Nikam, T.D., "Decolorisation and Detoxification of Widely Used Azo Dyes by Fungal Species Isolated from Textile Dye Contaminated Site", *International journal of current Microbiology and Applied Sciences*, 4, 4, 2015, pp. 813-834.

[12] Maier, J., Kandelbauer, A., Erlacher, A., Cavaco-Paulo, A., and Gübitz, G. M., "A New Alkali-Thermostable Azoreductase from *Bacillus sp*. Strain SF", *Applied and Environmental Microbiology*, 70, 2004, pp. 837-844.

[13] Mohandass, Ramya., Bhaskar, Anusha., Kalavathy, S., and Devilaksmi, S., "Biodecolorization and biodegradation of Reactive Blue by *Aspergillus sp.*", *African Journal of Biotechnology*, 6, 2007, pp. 1441-1445.

[14] Mahdavi, Talarposhti, A., Donnelly, T., and Anderson, G. K., "Colour removal from a simulated dye wastewater using a two-phase anaerobic packed bed reactor", *Water Res*, 35, 2001, pp. 425-432.

[15] Moorthi, S. P., Selvam, P. S., Sasikalaveni, Murugesan, A. K., and Kalaichelvan, P. T., "Decolorization of textile dyes and their effluents using white rot fungi", *Afr. J. Biotechnol.*, 6, 4, 2007, pp. 424-429.

[16] Mohammed, H. A., "Biodeclorization of Congo red Dye by using two species of Fungi", *Mesopotamia Environmental Journal***,** 1, 4, 2015, pp. 50-56.

[17] Nosheen, S., Nawaz, R., Arshad, M., and Jamil, A., "Accelerated biodecolorization of reactive dyes with added nitrogen and carbon sources", *Int. J. Agric. Biol*, 12, 2010, pp. 426-430.

[18] Pandey, A., Singh, P., and Iyengar, L., "Bacterial decolorization and degradation of azo dyes", *International Biodeterioration and Biodegradation*, 59, 2007, pp. 73-84.

[19] Ponraj, M., Gokila, K., Zambare, V., "Bacterial decolorization of textile dye-orange 3R, *International journal of advanced biotechnology and research*, 2, 2011, pp. 168-177.

[20] Ponraj, M., Jamunarani, P., and Zambare, V., "Isolation and Optimization of Culture Conditions For Decolorization of True Blue Using Dye Decolorizing Fungi', *Asian J. Exp. Biol. Science*, 2, 2, 2011, pp. 270-277.

[21] Qin, J. J., Htun, M., and Kekre, K. A., "Nanofiltration for recovering wastewater from a specific dyeing facility", *Separation and Purification Technology*, 56, 2007, pp. 199–203.

[22] Robinson, T., McMullan, G., Marchant, R., and Nigam, P., "Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative", *Bioresour Technol*, 77, 2001, pp. 247-255.

[23] Selvam, K., Swaminathan, K., and Chae, KS., "Microbial decolorization of azo dyes and dye industry effluent by *Fomes lividus", World J. Microbiol. Biotechnol.*, 19, 6, 2003, pp. 591-593.

[24] Vidali, M., "Bioremediation–an overview", *Pure Application Chemistry*, 73, 7, 2009, pp. 581–587.

[25] Zollinger, H., "Colour Chemistry –Synthesis, Properties and Applications of organic Dyes and Pigments", *VCH Publishers New York*, 1987, pp. 92-100.