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Research article

DECOLOURIZATION OF SELECTED PROCION DYE USING FUNGI, ACREMONIUM CHRYSOGENUM A COMPARISON WITH PHYSICAL ADSORBENTS

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ABSTRACT: Azo dyes, based on aromatic amines, may contain these amines as impurities introduced during the manufacturing process. Aromatic amines may also be present as a result of thermal or photochemical degradation of azo dyes. The more stable derivatives contain two aryl groups**.** As a consequence of π-delocalization, aryl azo compounds have vivid colors, especially reds, oranges, and yellows. Therefore, they are used as dyes, and are commonly known as azo dyes, an example of which is Disperse Orange. Some azo compounds, eg. methyl orange, are used as acid-base indicators due to the different colors of their acid and salt forms. The development of azo dyes was an important step in the development of the chemi azo colorants range in shade from greenish yellow to orange, red, violet and brown. The colours depend largely on the chemical constitution, whereas different shades rather depend on the physical properties. However, the important disadvantage limiting their commercial application is that most of them are red and none are green. The inoculum was prepared by adding 10ml of saline to the culture tube, which contain the *Acremoniumchrysogenum.* The present study designed to study the degradation of common laboratory dyes using fungi, acremonium chrysogenum. The dye selected was procion red. The selected dye procion red can be toxic in its secondary reactive form so there is a great need of them to be removed from the environment.

Key words: Aromatic Amines, Azodye, *Acremoniumchrysogenum,* Procion

INTRODUTION

Azo compounds are compounds bearing the functional group R-N=N-R', in which R and R' can be either aryl or alkyl. The N=N group is called an azo group, although the parent compound, HNNH, is called diimide. The more stable derivatives contain two aryl groups. The name azo comes from *azote,* the French name of nitrogen that is derived from the Greek *a* (not) + *zoe* (to live).

As dyes and pigments

As a consequence of π-delocalization, aryl azo compounds have vivid colors, especially reds, oranges, and yellows. Therefore, they are used as dyes, and are commonly known as azo dyes, an example of which is Disperse Orange 1. Some azo compounds, eg. Methyl orange, are used as acid-base indicators due to the different colors of their acid and salt forms. The development of azo dyes was an important step in the development of the chemical industry.

Aryl azo compounds

Aryl azo compounds are usually stable, crystalline species. Azobenzene is the prototypical aromatic azo compound. It exists mainly as the trans isomer, but upon photolysis, converts to the cis isomer. Aromatic azo compounds can be synthesized by using an azo coupling reaction, which entails an electrophilic substitution reaction where an aryl diazonium cation attacks another aryl ring, especially those substituted with electron-releasing groups.1. Since diazonium salts are often unstable near room temperature, the azo coupling reactions are typically conducted near ice temperatures. The oxidation of hydrazines (R-NH-NH-R') also gives azo compounds. (Clarke E. A. and Anliker R, 1980). Azo dyes derived from benzidine are carcinogens; exposure to them has classically been associated with bladder cancer. (Golka K, et al 2004) accordingly, the production of benzidine azo dyes was discontinued in the 1980s "in the most important western industrialized countries" (Hunger K. and Jung R, 1991).

Azo dyes

Azo dyes are, due to their relative simple synthesis and almost unlimited numbers of substituents, the most numerous group of synthetic dyes. Azo dyes do not occur naturally. Azo dyes may have one or more azo groups. Azo dyes with one azo group are called mono azo dyes, with two azo groups, diazo dyes, followed by triazo and polyazo dyes. Azo dyes with more than three azo linkages are designated polyazo dyes. The most commercial important are mono- and diazo dyes, triazo dyes, whereas polyazo are much less important.

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Technical properties of azo dyes

Azo dyes represent the largest, in number, group of synthetic dyes and the most widely, in tonnage, manufactured. These dyes are, compared to natural dyes, better capable of meeting requirements regarding technical properties, e.g. fastness to light.

The chemical diversity of azo dyes permits a wide spectrum of shades, mainly within the scale of red. A disadvantage limiting their application is, however, that none of the azo dyes are green. The great majority of azo dyes are water soluble and they colour different substrates by becoming physically attached. The attachment may be due to adsorption, absorption or mechanical adherence. Azo dyes have a broad industrial application field. They are used for colouring of synthetic and natural textile fibres, plastics, leather, paper, mineral oils and waxes. Their abilities of keeping an intense colour and fastness to light are quite good in most cellulose fabrics but are relatively poor in colouring of cotton and wool. A number of azo dyes are used as food colorants in cosmetics and as drugs for treatment of bacterial infections. Most of the commercial available azo dyes are in fact formulations of several components in order to improve the technical properties of the dyeing process. The content of a specific dye lies in the range of 10 to 98%. The grouping of dyes, including azo dyes, often reflects a strict defined concept of application. There is a strong evidence that aromatic amines require metabolic activation for carcinogenicity. The first step involves N-hydroxylation and N-acetylation, and the second step involves O-acylation yielding acyloxy amines. These compounds can degrade to form highly reactive nitrenium and carbonium ions. These electrophilic reactants may readily bind covalently to genetic material, namely cellular DNA and RNA. (Brown M. A. and DeVito S. C , 1993) This process may induce mutations, and it is recognised that mutations can lead to formation of tumours. Although the primary acute hazard associated with exposure to aromatic amines is carcinogenesis, methemoglobinemia is attributed to the same mechanism of metabolic activation.

Problems of impurities: Several impurities may be found in almost all commercial available azo dyes. Impurities may be introduced during the manufacturing processes or during the storage.

REVIEW OF LITERATIRE

Azo dyes, based on aromatic amines, may contain these amines as impurities introduced during the manufacturing process. For example, azo dyes based on benzidine or *o*-toluidine may contain residues of benzidine or *o*-toluidine, respectively, used as intermediates in the manufacturing process. Aromatic amines may also be present as a result of thermal or photochemical degradation of azo dyes. It is known, that sunlight may cause release of 1-aminonaphthalene formed azo dyes based on this amine.(Brown M. A. and DeVito S. C, 1993).

Exposure: Exposure to azo dyes also entails exposure to the component aromatic amines due to: breakdown of azo dyes. Presence of aromatic amines as impurities (their intermediates or breakdown products). Exposure to aromatic amines is of great concern, as many of them are characterized by having serious long-term effects. Exposure to azo dyes may take place through inhalation and accidental ingestion. Absorption of azo dyes through the skin is rather doubtful, whereas the aromatic amines may be absorbed. Non-occupational exposure to azo dyes may take place by the wearing of coloured textiles and by playing with colored toys which not conform to requirements and standards harmonized at the European level by the Council Directive concerning safety of toys. Inhalation of cigarette smoke represents the greatest non-occupational exposure, as the smoke contain aromatic amines along with many other hazardous compounds. It is known that inhaled cigarette smoke enhance the incidence of bladder cancer, and heavy cigarette smoking doubles the risk of getting bladder cancer. (Cartwright R. A, 1983). An important natural abiotic degradation mechanism is photolysis and hydrolysis as a function of pH in the range of pH 4-9. The evidence of the role of hydrolysis in degradation of azo dyes is not conclusive. (ETAD, 1989). The photo-stability of azo dyestuffs is high in pure water but in the pre- sence of natural humic materials, the photo decomposition is strongly accelerated, probably through oxidation by single oxygen or oxy-radicals (Brown D. and Anliker R, 1988). Other advanced oxidation processes include Fenton's reagent and TiO_2 photo-oxidation (Shu H.Y, 1994). It is assumed that the main abiotic removal mechanism for dyestuffs in wastewater treatment plants is adsorption of sludge. However, other effects like sedimentation, precipitation or flocculation may also play a role.[9]Formation of azo compounds by oxidative coupling has been demonstrated in aerobic enrichment cultures from the aromatic amines (Field J.A, 1995).

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MATERIALS AND METHODS

Preparation of standard graph for procion red

10mg of procion red was weighed and dissolved in 10ml of distilled water.1ml of the prepared solution was taken in another test tube and diluted with 9ml of distilled water. Then it was distributed into 10 test tubes in different concentrations that is 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5ml. The volume in the test tubes were made up to 5ml with distilled water and a test tube with 5ml of distilled water was used as a blank to set the colorimeter to zero. The readings were taken at 660nm against the blank. A standard graph was prepared by taking concentration of malachite green on X-axis and O.D values on Y-axis.

Preparation of medium: Composition of media (100ml)

Sucrose : 3g
$NaNO_3$: 0.3g
KCl : 0.05g
$MgSO_4$: 0.05g
K_2HPO_4 : 0.1g

Media preparation

Media was prepared by dissolving the components mentioned above one by one in distilled water and the final volume was made to 600ml.The media was equally distributed into 12 conical flasks that is each with 50ml of media. Then the media was autoclaved at 121˚c and 15 lbs for 15min.Then the media was cooled to room temperature.

Preparation of inoculum and inoculation: The inoculum was prepared by adding 10ml of saline to the culture tube, which contain the *Acremonium chrysogenum*. The spores are then absorbed into the saline. 0.5 ml of inoculum was added to each conical flask and they were placed on shaker at 150rpm and the growth was observed after 48hours.

Optimization of dye concentration

10mg of procion red was weighed and dissolved in 10ml of distilled water.1ml of the prepared solution was taken in another test tube and diluted with 9ml of distilled water. Then the dye was autoclaved at 121^0C and 15 lbs for 15min cooled to room temperature. Immediately after the addition of dye, 1.5ml of media was taken out from each conical flask and transferred to 12 eppendorff tubes and the conical flasks were again placed on shaker at 180rpm.The eppendorff tubes were centrifuged at 10,000rpm for 10min and the supernatant was carefully separated from the tubes using micropipettes. Absorbance was measured at 650nm.

Optimization of carbon source

Prepared 250 ml of medium and divided into five parts. Sucrose in different concentrations starting from 0.5 to 2.5 gm was added to above fractions of medium and autoclaved. After cooling the dye was added in equal quantity to all the flasks and allowed for incubation. The reduction in dye concentration at different incubation periods was recorded at 650 nm.

Optimization of nitrogen source

Prepared 250 ml of medium and divided into five parts. $NaNO_3$ in different concentrations starting from 0.5 to 2.5 gm was added to above fractions of medium and autoclaved. After cooling the dye was added in equal quantity to all the flasks and allowed for incubation. The reduction in dye concentration at different incubation periods was recorded at 650 nm.

Decolorization using saw dust

Prepared 250 ml of medium and divided into five parts. Saw dust in different concentrations starting from 0.5 to 2.5 gm was added to above fractions of medium and autoclaved. After cooling the dye was added in equal quantity to all the flasks and allowed for incubation. The reduction in dye concentration at different incubation periods was recorded at 650 nm.

Decolorization using egg shell

Prepared 250 ml of medium and divided into five parts. Egg shell in different concentrations starting from 0.5 to 2.5 gm was added to above fractions of medium and autoclaved. After cooling the dye was added in equal quantity to all the flasks and allowed for incubation. The reduction in dye concentration at different incubation periods was recorded at 650 nm.

RESULTS

Present result study the degradation of common laboratory dyes using fungi, Acremonium chrysogenum..In the first step dye concentration were optimized and have shown a maximum degradation at 400ug (Table 1-3). Degradation was studied under different concentrations of carbon source i.e 0.5 to 2.5 gm.

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Maximum degradation was obtained at o.5gm concentration where the left over dye concentration is just 30ug. When optimizing the same with nitrogen source the maximum degradation was seen 0.5gms (table 4). Decolorization studies were also carried out by using egg shell and saw dust for a period of 6hrs. The maximum decolorization was seen with 0.5gm of saw dust and 0.5 and 2.5 gm of egg shell (Table 5, 6). Microbial decolorization of procion redhas been investigated by a few authors. They have studied the decolorization of procion yellow by *Acremonium kiliense*. According to them 95.4% PY was decolorized within 72 h when the concentration of the dye was 5 mg L-1 but decolorization was only 35.48% when the dye concentration was doubled. They have attributed this trend to be due to inhibition of fungal growth at high dye concentration. According to them 50 mg L-1 MG was com pletely decolorized under static anoxic conditions within 5 h by bacteria *K. rosea* MTCC 1532; however decolorization was not observed at shaking condition. Scientist investigated the decolorization of different dyes including malachite green by *Aeromonas hydrophila*. According to them 50 mg L-1 MG was 90% decolorized within 10 h under aerobic culture conditions. Decolorization of malachite green by *Citrobacter* sp. According to their results, 100 _M MG was 80 % decolorized within 1 h. The present study has revealed that Humicola is able to decolorize 100 malachite green and methyl red by 97.43 and 96.91% respectively. Dye concentration in the range of up to 0.6 mg had no significant effect on % decolorization in case of both the species. It is also clear that C source in the decolorization medium has significant effect on % decolorization in both cases.

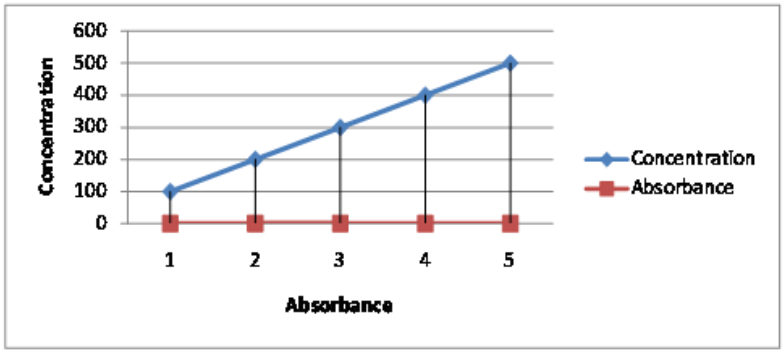

Fig-1 Standard curve for procion red

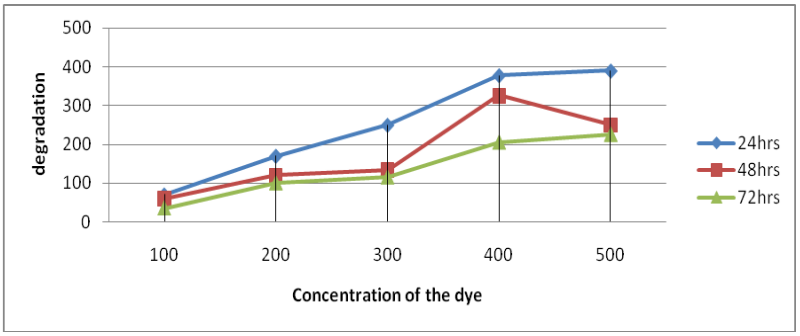

Fig-2 Optimization of dye concentration

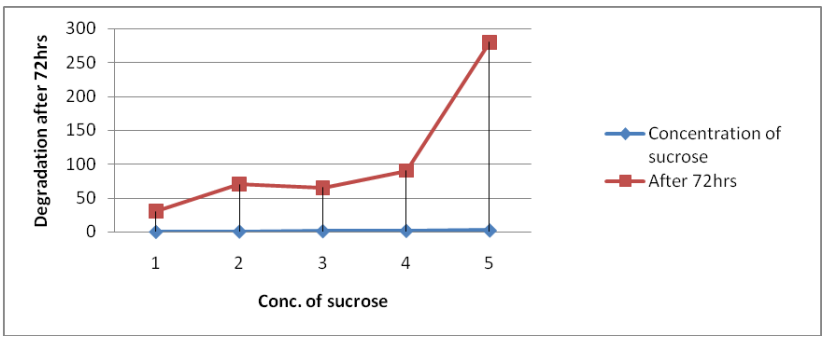

Fig-3 Optimization of sucrose

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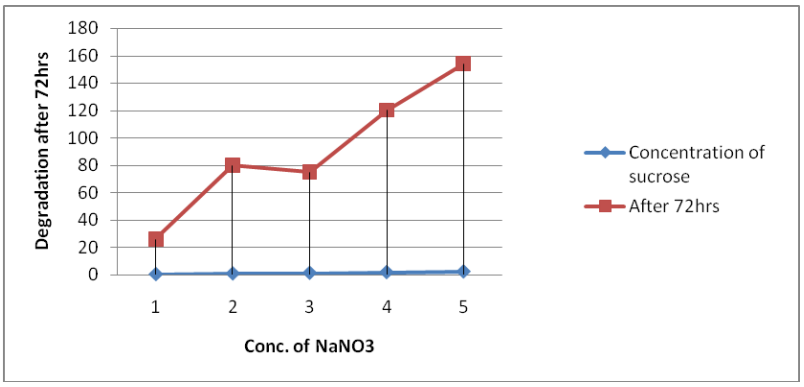

Fig-4 Optimization of $NaNO_3$

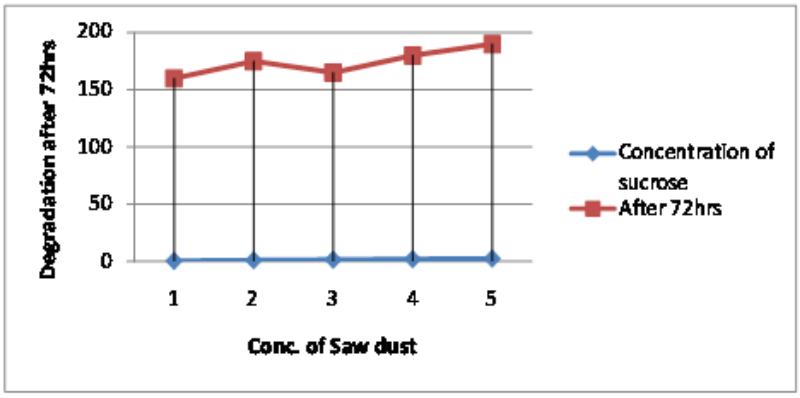

Fig-5 Degradation using saw dust

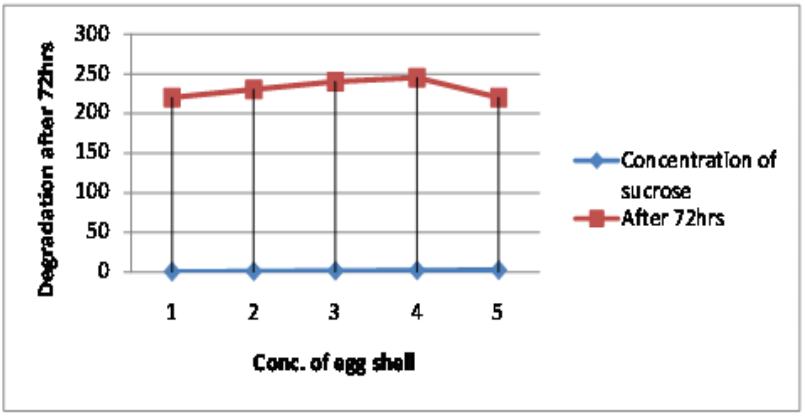

Fig-6 Degradation using egg shell

Table 1: Preparation of standard curve of procion red

Concentration in ug	After 24hrs	After 48hrs	After 72hrs
100	70	60	35
200	170	120	100
300	250	134	115
400	378	326	205
500	390	250	225

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Table 2: Optimization of dye concentration

Concentration of the dye in ug	Absorbance at 660nm
100	0.3
200	0.6
300	0.9
400	0.12
500	0.17

Table 3: Degradation with sucrose optimization

Concentration in ug	Concentration of sucrose gm	After 72hrs
400	0.5	30
400	1.0	70
400	1.5	65
400	2.0	90
400	2.5	280

Table 4: Degradation with $NaNO_3$ optimization

Concentration in ug	Concentration of $NaNO_3$ gm	After 72hrs
400	0.5	26
400	1.0	80
400	1.5	75
400	2.0	120
400	2.5	154

Table 5: Degradation with saw dust

Concentration in ug	Concentration of Saw dust gm	After 6 hrs
400	0.5	160
400	1.0	175
400	1.5	165
400	2.0	180
400	2.5	190

Table 6: Degradation with egg shell

Concentration in ug	Concentration of egg shell gm	After 6 hrs
400	0.5	220
400	1.0	230
400	1.5	240
400	2.0	245
400	2.5	220

DISCUSSION

The present study designed to study the degradation of common laboratory dyes using fungi, Acremonium chrysogenum. The dye selected was procion red To carry out the degradation studies standard curve for selected dye was prepared at definite concentrations and the curve was straight line passing through origin at 45 degree angle Degradation was studied under different concentrations of carbon source i.e 0.5 to 2.5 gm. Maximum degradation was obtained at o.5gm concentration where the left over dye concentration is just 30ug. When optimizing the same with nitrogen source the maximum degradation was seen 0.5gms. Decolorization studies were also carried out by using egg shell and saw dust for a period of 6hrs.

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CONCLUSION

The selected dye procion red can be toxic in its secondary reactive form so there is a great need of them to be removed from the environment. In the present study an attempt has been made for its removal using a fungi *Acremonium chrysogenum.* Better degradations were obtained by changing different concentrations of carbon and nitrogen sources. Even better degradations might be obtained through physical adsorbents like saw dust and egg shell. So it can be concluded that the study has to be further progressed by adapting different mutational processes and can be effectively applied for degradation. But all these require extensive studies.

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