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BIOSORPTION OF PARAPHENYLENE DIAMINE IN BLACK HAIR DYE USING PRETREATED AND UNTREATED *A. NIGER* BIOMASS

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Abstract

PPD is used in almost every hair color in the market, regardless of brand. It is an arylamine, and is one of the most common allergens and has pronounced mutagenecity. Uptake of PPD was noted at 400 nm at room temperature in different media as well as in saline (pH 2.0 to 6.0). The biomass was subjected to some selective pretreatments with 0.1M NaOH, NaOH with autoclaving,1M bicarbonate, 5N HCl, heat shock (100^0 C for 5min), acetone, acetic acid, ethanol and 1% SDS. Uptake of PPD using pretreated biomass was carried out at 100 µg/ml in citrate – phosphate buffer, pH 2.0. 76 - 100% uptake was noted after NaOH and Ethanol pretreatments. No uptake was noted after HCl pretreatment. The dye recovered after treating biomass with methanol following dye uptake was 14 -71% .The elute had a λ_{max} of 400 nm indicating that the dye was not transformed but only biosorbed.

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INTRODUCTION

Paraphenylene diamine is an arylamine, and is one of the most common allergens among patients with allergic contact dermatitis [1]. Para-phenylenediamine is a colourless/slightly pink, grey or yellow crystalline solid (lumps or powder). On oxidation, usually through exposure to air, it turns red, brown then finally black. PPD is essentially a dye and chemical intermediate.(Farrow,2002). It is used in almost every hair color in the market, regardless of brand. Even the so-called "natural" and "herbal" hair colors, may be ammonia-free but contains PPD. PPD is a preferred chemical due to its low toxicity levels and its ability to withstand high temperatures and retain its stability. It is good for hair dyes because it produces a natural color which doesn't fade as readily with washing and drying. PPD itself is colorless , it gains its color once it's exposed to oxygen.

PPD, apart from being used primary intermediate in hair dye formulations [2] is also used as dyes for fabrics, fur etc. This compound is also used as photographic developing agent and as an intermediate in the manufacture of azo dyes, antioxidants, and accelerators for rubber vulcanization [3].Allergic reactions to PPD range from mild dermatitis through to more severe dermatitis, swelling of head, eyes and face, anaphylactic reactions and death.

MATERIALS AND METHODS

Screening of fungi growing in presence of black hair dye:

Fungal isolates were screened after enrichment of soil and cowdung in Czapekdox broth [5] containing 50 µg/ml of the balck hair dye. After 7 days of enrichment, loopfuls were transferred on Czapekdox agar medium. Commercially available black dye was used. Fungi that developed after 5 to 7 days were transferred on Czapekdox and Potato dextrose agar [5] slants. Seven fungal cultures were selected for comparative dye uptake studies. Cultures were identified based on colour, colony characters, spore and mycellial presentation etc. The dye stock solution was prepared by dissolving it in 1ml of ethanol, swirling the solution till it dissolved completely and further diluted as required with distilled water. All dye solutions, media etc were sterilized by autoclaving before use. The cultures selected were grown on Czapekdox medium and fully grown agar blocks were cut using a sterile steel borer (8mm). Ten mycellial plugs were directly inoculated in Czapekdox medium along with 50

µg/ml of black hair dye containing PPD. Dye decolorisation was noted every day visually in order to screen the best isolate in comparison with uninoculated controls.

Dry weight measurements in presence of the dye:

The ability of the cultures to decolorize the dye was detected visually followed by spectrophotometry at 400 nm (λ_{max}) and dry weight measurements as per standard procedures [6].

Effect of different sugars and media on growth and dye uptake:

A.niger was grown on Potato dextrose agar. Three mycelial plugs (8mm diameter) were inoculated in different media. The pH of Minimal medium [7] was adjusted in a range of 4.0 to 7.0 using 1N HCl or 1N NaOH. Dye uptake was noted at a concentration of 100µg/ml. Absorbance of the residual dye was noted at 400 nm (as determined from the spectral scan) using a photocolorimeter (Elico c157) after 7 days incubation at room temperature and compared with abiotic controls.

S**uitable pretreatment of the biomass and dye uptake thereafter:**

Fungal biomass preparation:

The culturing methods, media for growth and different pretreatments that have been used are as per the methods adopted by Fu and Viraraghavan [8]. The fungal biomass was separated by filtering the growth media through a whatmann filter paper followed by washing with generous amounts of distilled water. Biomass was grown in Potato dextrose broth containing three mycelial plugs of 8mm diameter that allowed biomass formation equal to a dry weight of 0.260 g.(7days incubation at room temperature). The harvested biomass was pretreated as mentioned in table 4. The suitably pretreated biomass was washed with liberal amounts of distilled water until the pH of the wash solution was close to pH = 6.0. Then the pretreated biomass was autoclaved for 20 min at 121°C and oven dried at 50 to 60°C for 24 to 48 h. The dry biomass was powdered using a mortar and a pestle. Dye uptake studies were carried out at100µg/ml in citrate –phosphate buffer pH 2.0. Absorbance at 400 nm was noted after 3 days of uptake at room temperature.

Effect of different concentrations of NaOH on pretreatment of the biomass:

Biomass was further treated with different concentrations of NaOH (0.1, 1and 5N) as before. The biomass was repeatedly washed and finally used for dye uptake at different pH.

Effect of pH on dye uptake:

Out of the several pretreatments carried out, the highest uptake was noted with the biomass pretreated with NaOH. Thus dye uptake using this treated biomass was noted at 50µg/ml in saline adjusted to different pH.(2 to 6).

Use of pretreated biomass for dye uptake:

Further pretreatment was carried out at increasing concentrations of NaOH such as 10, 20, 25 N and also by use of methanol .Dye uptake was determined using these treated biomass at 50 µg/ml in saline pH 2.0.

RESULTS AND DISCUSSION

Visual reduction in color in comparison to uninnoculated controls were noted as in table 1.Reduction in colour was significant with respect to cultures R4,A1 and A2

Table:1.Dye decolorisation demonstrated by screened isolates.

Serial no.	Cultures	Reduction in color.
1	R 1	-
2	R 2	-
3	R 3	-
4	R 4	+++ (significant)
5	A 1	++
6	A 2	++
7	CD1	-

It may be observed from table 2 that there was a reduction in dry weight with respect to R2, R3,R4 and CD1. Culture that showed highest dry weight was R1 followed by A1. If cultures are able to maintain a higher dry weight when growing in presence of the dye exclusively in comparison to other cultures ,it may indicate their ability to use the dye as a source of carbon, nitrogen or both.

Table 2: Dry weight measurement of isolates in presence of hair dye.

Serial number	**Culture**	**Dry weight (g)**
1	R 1	0.680
2	R 2	0.300
3	R 3	0.320
4	R 4	0.320
5	A 1	0.580
6	A 2	0.460
7	CD 1	0.190

Table 3: Effect of media composition on growth and PPD uptake.

Media	Dry weight g/50ml	% uptake
Glucose minimal media, pH 4.0.0	0.311	13.28
pH 5.0	0.825	22
pH 6.0	1.84g	47.36
pH 7.0	2.130	82.5
Minimal media with sucrose, pH 4.0	0.034	12
pH 5.0	0.230	19
pH 6.0	0.640	23
pH 7.0	1.07	45.48
Potato dextrose broth, pH 4.0.	0.654	32
pH 5.0	1.282	44.2
pH 6.0	1.562	68.12
pH 7.0	1.433	80

It was noted that the highest uptake was in Glucose minimal medium and in potato dextrose broth at pH 6.8.(Table 3). The extent of decolorisation depends on the nature of the carbon source. This is probably related to differences in bacterial metabolism when given either glucose or glycerol [9]. The differences in energy output, formation of different metabolic intermediates (organic acids, dye chellators) as a consequence of employing different substrates through metabolic pathways would have an effect on growth and therefore on dye decolorisation. Ramalho *et al*.[10] noted that glucose concentrations of up to 20 mM in 0.05 M phosphate buffer (pH 4.0) improved conditions for the aerobic reductive decolorisation of azo dyes.

The effects of sodium hydroxide treatment in preparation of fungal biosorbent was better than the others used.

Table 4:Dye decolorisation after pretreatments of biomass.

Pretreatment methods	Duration	% uptake	% dye released from mycelia
0.1M NaOH	12 h	92.85	35.71
0.1M NaOH with autoclaving	12h	100	14.28
1M Bicarbonate	12h	92.85	0
Autoclaving	15lbs/20 min	100	0
Heat shocking at 100^0 C	5 min.	92.85	0
5N HCl	12 h.	42.85	0
1% SDS	12 h.	100	14.28
Acetone	5 min.	100	71.00
Acetic acid	12h.	65.75	57.14
Ethanol	5 min.	100	28.57

Table 5: Effect of pH on dye decolorisation

pH	% uptake	% dye released from mycelia (Elution)
2.0	73.00%	30.76
3.0	53.84%	38.46
4.0	62.50%	87.50
5.0	16.39%	18.30
6.0	38.46%	50.00

It may be observed from table 5 that the uptake at pH 2.0 was the highest. The uptake dropped at pH 3.0 and pH 5.0 on account of a lesser biomass. Since the conditions are unsupportive and pH dependent it is likely to be biosorption. It has been reported by Bidisha et al.[11] that a fungus, *Aspergillus foetidus*, was found to utilise azo reactive dye(s) such as drimarene red under active growth and growth unsupportive conditions. The key factors that influence the process of biosorption of dye in growth non-supportive medium are pH, temperature, and age and concentration of fungal biomass. The presence of 0.1% azide and phosphate in the fungal growth medium causes displacement of bound dye from the biomass.

Table 6: Pretreatment of biomass using various concentrations of sodium hydroxide:

Pretreatments	% uptake	% dye released from mycelia
10N NaOH	100	78.57
20N NaOH	100	50.00
25N NaOH	100	57.14
Methanol	70	100

It may be observed from table 6 that an increase in sodium hydroxide concentration shows a constant uptake .The extent of elution using methanol was more in case of biomass treated with 10N NaOH and therefore the preferred concentration of NaOH for pretreatment. Bidisha et al.[10] reported that sodium hydroxide is an effective agent for the leaching of dye from the loaded fungal biomass. The results suggest the possibility of applying the isolated fungus for decolourisation of textile mill wastewater. Biosorption using dead cells has many advantages over the live cell systems. Application of dead macro fungus would not only heap in the advantages resulting from the application of dead cells but would also overcome the possible limitations arising out of the poor engineering properties of other biomasses.[12].

Significant findings:
The dye was being used as a nitrogen source since the media did not contain any added nitrogen source. Biosorbents prepared by pretreatments with NaOH showed very good dye uptake . Elute had the same maxima as that of PPD. This means that there was extracellular accumulation .which was released by washing the loaded biomass with methanol. Thus *Aspergillus niger* was able to show dye decolorisation in live as well as dead cell biomass.

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