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DECOLORIZATION OF OFFSET PRINTING INDUSTRY EFFLUENT IN A MIXED BED REACTOR USING A BACTERIAL CONSORTIUM

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ABSTRACT

A bacterial consortium consisting of two isolated strains (EPPI3 & DPPI2) was developed for the degradation of mixture of two dyes, Scarlet red and Magenta present in offset printing industry effluent at pH7 and an optimum temperature 30°C.The degradation efficiency of the consortium in different environmental conditions (pH, temperature, agitation, carbon and nitrogen sources) and different effluent concentration were studied. Further with all optimized conditions effluent was treated in Mixed Bed Reactor (MBR).The physicochemical analysis using standard methods as well as metals by Atomic Absorption Spectroscope and the analysis of degraded products by HPLC, LCMS were carried out. The given microbial treatment to the dyes observed in effluent of offset printing industry showed complete mineralization and only presence of some untraceable, negligible very low molecular weight compounds were found.

INTRODUCTION

The age of industrialization, information and growing economy has boosted the development of printing industry, one such overlooked industry is "Offset Printing". After completion of printing process the printing machines and other equipments are washed by using water and clean- up reagents, which generate effluent. Color is one of the most oblivious indicators of water pollution and the discharge of highly colored synthetic dyes effluent can be damaging to the receiving water bodies (Nawar and Doma, *et al*. 1995) and disturbing the ecological balance. It is very difficult to treat the effluents from textile and printing industries by the commonly used physical and chemical methods mainly because of its high BOD, COD, heat, colour, pH and presence of metals (Resmi and Abraham, 2004). Therefore, over last two decades , interest has been focused on microbial biodegradation of dyes in an effluent as a viable alternative (Arora *et al*. 2005) and the effectiveness of microbial degradation depends on the survival , adaptability and activity of the selected organism (Resmi, *et al*. 2004; Cripps, *et al*. 1990 and Pasti, *et al*. 1992). So the microbial consortia are used as black boxes without analyzing the constituent microbial populations for environmental remediation. The complexity of microbial consortium enables them to act on a varity of pollutants (Resmi, 2004).

The main objective of the present investigation was to isolate, identify and optimize the effective microorganisms (EM) to develop a consortium, which

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was further used to develop a cost effective, ecofriendly microbial technology for effluent treatment.

MATERIALS AND METHODS

Effluent Collection

Offset printing industry effluents were collected from five major printing industry outlets within Latur city (M.S).

Chemicals Used

All chemicals used for microbiological & chemical study were of standard analytical grade.

Enrichment, isolation and screening of effective microorganisms (EM)

Effluent samples collected from different industry outlets were enriched in presterilized nutrient broth medium for 24h.The enriched culture was spread on nutrient agar plates. The morphologically distinct bacterium was isolated and used for the decolorization studies i.e. screening of effective microorganisms (EM).

In 250 mL Erlenmeyer flask, containing 50mL presteriliszed nutrient broth with 2 mL of effluent. The flasks were inoculated with a loop full of cell growth from the agar plates and incubated in incubator (Kumar's bacteriological incubator-KI-05-03) at static manner for 24h at 28°±1°C.Screening was based on percent decolorization and time required for decolorization.

Development of a Consortium (BM)

On the basis of the result of screening, three bacterial isolates were selected and identified methods as *Bacillus cerus*, *Bacillus subtillis* and *Micrococcus indicus* (accession number-EF 432780, NCCS Pune) by standard methods. Further were labeled as EPPI3, KPPI3 and DPPI2 respectively. The selected organisms then were mixed in different combinations. The bacterial combination that showed maximum decolorization in short time period was selected as potent consortium for the further studies for effluent treatment.

Assay of Decolorization

An aliquot was centrifuged at 8000 rpm for 15 min to separate cell mass. Supernatant was used to determine decolorization by measuring change in absorbance at λmax 327nm quantitatively using UV-visible spectrophotometer (Elico Ltd.)

The percentage decolorization was calculated as follows-

$$\% \text{ Decolorization} = \frac{\text{Iabs} - \text{Fabs} \times 100}{\text{Iabs}}$$

Iabs - Initial Absorbance
Fabs - Final Absorbance

All the assays were performed in triplicate and compared with un-inoculated controls.

The degradation was monitored by High Performance Liquid Chromatography (HPLC). After complete decolorization by consortium (BM), culture broth was centrifuged at 12000 rpm for 30 minutes and equal volume of ethyl acetate was used for extraction. The extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness in rotary evaporator. The crystals obtained were dissolved in small volume of HPLC grade methanol and used for analysis. HPLC (Simadzu) was carried out on column (YMC ODS, 150 mm x 4.6 mm, 5- µ, ID: E-AC-1/07/COL/22) with 0.05 % TFA (Tri fluro acetic acid) in acetonitrile as mobile phase at flow rate of 1.4 mL/min at wavelength 220 nm and performance of MBR, i.e. treated effluent by consortium results in visually decolorization and the degraded products were confirmed by using Liquid Chromatography and Mass Spectrum (LCMS) (Simadzu) on column (YMC ODC,50x4.6mm,3µ ID: E-AC-1/07/COL/26) with 0.05 % TFA (Tri fluro acetic acid) in acetonitrile as mobile phase at flow rate of 1.2mL/min at wavelength 220nm.

Optimization of Medium Conditions for Effluent Decolorization by Consortium (BM)

The decolorization potential of microorganisms in either pure form or consortium depends on the components of the medium and growth conditions (Arora, *et al*. 2007). Thus the effect of various nutritional and environmental parameters such as effect of pH, temperature, carbon and nitrogen sources, effluent concentration (initial dye concentration) and agitation etc. were studied.

The developed consortium (BM) was grown in 250 mL Erlenmeyer flask containing 50mL presterilized nutrient broth for 24h were used to monitor decolorization of effluent at 28°±1°C static manner and shaking on rotary incubator shaker for (40, 60, 80,100 & 120). Studies on effect of pH ranging from (2.5 to 10.5) and temperature ranging from (25° to 50°C) were carried out in nutrient broth as well as decolorization at different effluent concentrations (initial dye concentration) (2% to 10%) were studied by using consortium (BM). To study the effect of carbon and nitrogen sources on the decolorization of effluent, modified nutrient broth was used with 1% carbon sources such as glucose, fructose, xylose arabinose and 1% nitrogen sources like yeast extract, peptone, amonium nitrate and sodium nitrate. In all above parameters percent decolorization was measured by using previously given formula.

The screening of the effluent sample by LCMS and comparison study on HPLC was done under polar mobile phase. Though M+ for monomeric unit of dye is not observed in LCMS analysis however M+=550 for the fragmented dimeric unit a Scarlet red (ret. time-3.95) was observed in LCMS and molecular fragments of Magenta b or b' (M + 1 = 284), M + = 284 of c (ret. time 3.4) was observed in LCMS analysis indicates the presence of said dyes in the offset printing industry effluent.

To conclude the effluent consists of unconsumed mixture of Scarlet red, Magenta dye, some degraded organic aromatic fragments along with Kerosene and suspended particles in water.

Development of a Microbial Technology (Reactor Design)

The dye content in the effluent is relatively resistant to biodegradation. Though the decolorization is mainly based on physical or chemical methods, they have some drawbacks such as high cost, formation of hazardous by-products and intensive energy requirements (Kim *et al*. 2004).

Therefore microbial biodegradation is an acceptable alternative. To support this experimentally, a locally designed Mixed Bed Reactor (MBR) was used.

Use of consortium shows an interesting possibility for effluent treatment. MBR is designed in such a way that high microbial load, prolonged microbe retention time, cost effective and most important is the easy operation and control.

On the basis of the physicochemical analysis of all the five effluent samples only one effluent with all high values was used in this study for further treatment.

Inocula for the decolorization experiments were prepared by growing the bacterial cultures separately and combined in 1% modified nutrient broth.

For Mixed Bed Reactor (MBR), three 5 litre capacity containers, made with biologically and chemically inert material, having 38cm height and 20cm diameter were used.

The containers were packed with two layers of gravels and two layers of sand of 6cm and 5cm height respectively. The lower most layer was of fine sand followed by the layer of coarse sand. Third layer was of small gravels and the upper most layer of big gravels.

Three containers with all optimized conditions were arranged in such a manner that the treated effluent from the first container should pass to the second container and then to the third container through a pipe having a knob to control the flow rate.

Initially, the effluent is treated with KPPI2 in the first container for a specific time and then passed to the second container.

Now, the treated effluent received in the second container is treated with EPPI3 for a specific time and then passed to the third container.

In the third container, the treated effluent is again treated with the consortium of KPPI2+EPPI3 i.e. 'BM' for a specific time. Finally, the treated effluent is removed from the third container.

The total system was operated in recycle mode for better performance evaluation.

The bioreactor performance in these treatments was examined by comparing physicochemical characteristics, spectroscopic analysis as per the standard methods.

RESULTS

The decolorization performance of effluent by consortium (BM) was increased when the environmental conditions were optimized, i.e. 60% decolorization was observed at pH 7, 62.2% at 30°C temperature and 66.23%, 70.35% decolorization in presence of 1% glucose and 1% peptone respectively as carbon and nitrogen source.

100% decolorization was achieved both at 5%

Table 1. Decolorization of KPP waste effluent by selected effective bacterial isolates

Medium Used	Combinations Used	% Decolorization	Time Required For Decolorization
Nutrient broth	EPPI3 + DPPI3	57.29	24 hour
Nutrient broth	KPPI2 + DPPI3	50.15	24 hour
Nutrient broth	EPPI3 + KPPI2	50.28	24 hour

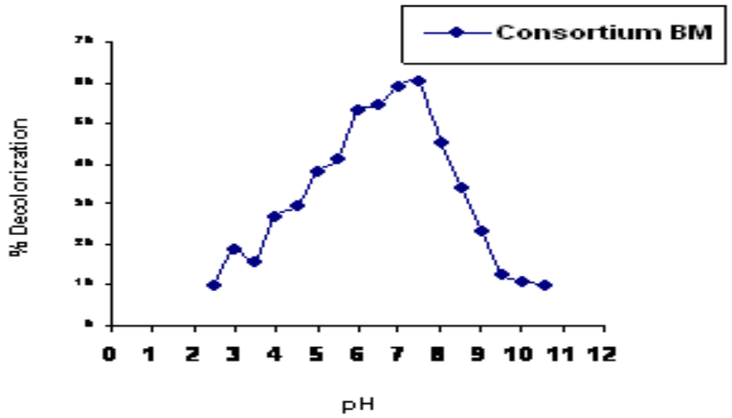

Fig. 1 Effect of pH on decolorization of KPP waste effluent by consortium (BM)

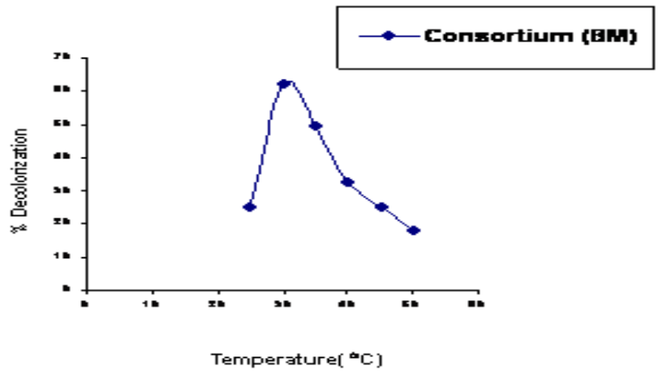

Fig. 2 Effect of temperature on decolorization of KPP waste effluent by consortium (BM)

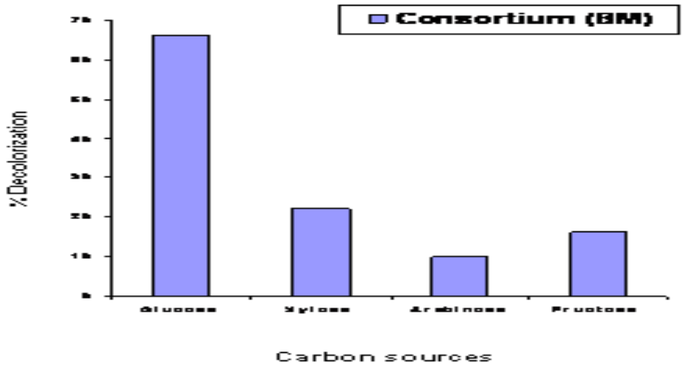

Fig. 3 Effect of carbon Sources on decolorization of KPP waste effluent by consortium (BM)

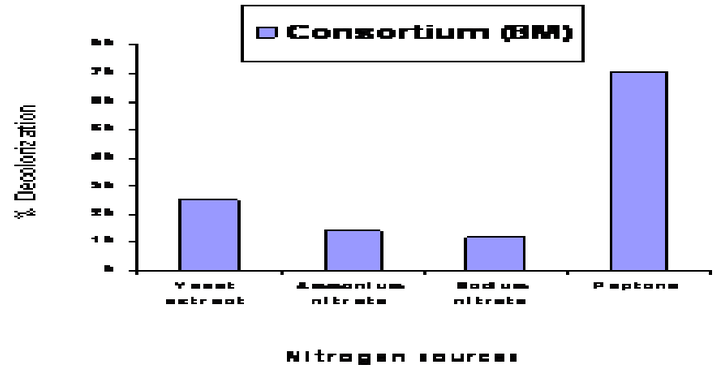

Fig: 4 Effect of nitrogen sources on decolorization of KPP waste effluent by consortium (BM)

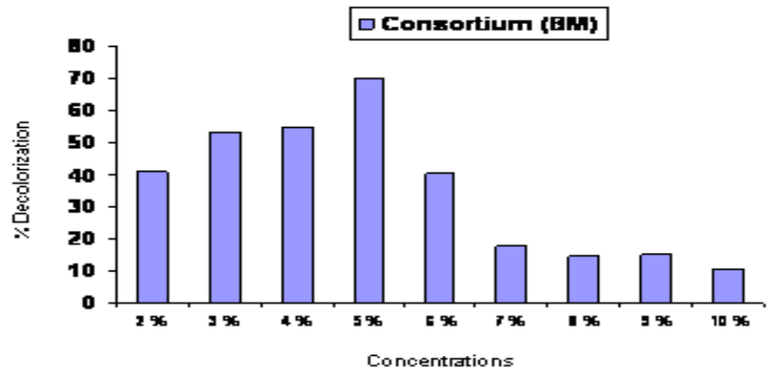

Fig. 5 Effect of effluent concentrations on decolorization of KPP waste effluent by consortium (BM)

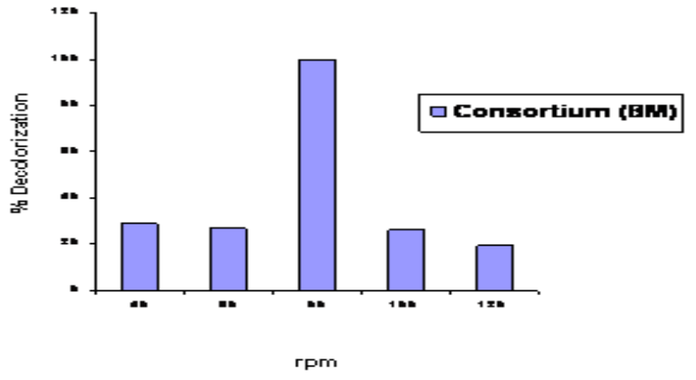

Fig. 6 Effect of agitation on decolorization of KPP waste effluent by consortium (BM)

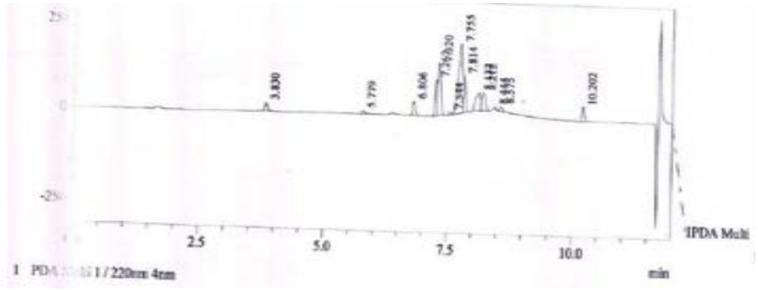

Fig. 7 HPLC analysis before treatment

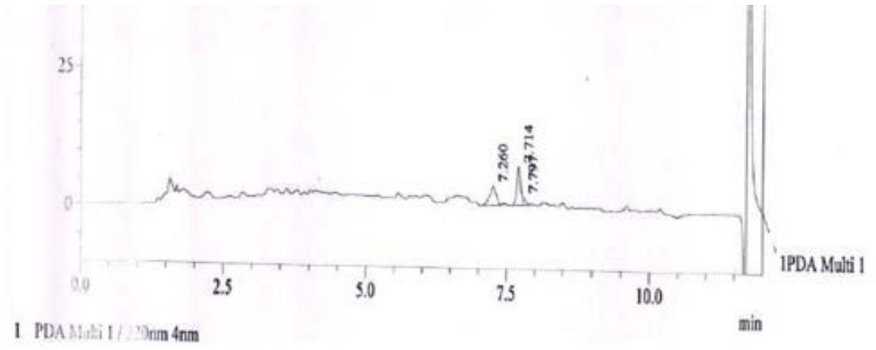

Fig. 8 HPLC analysis after treatment

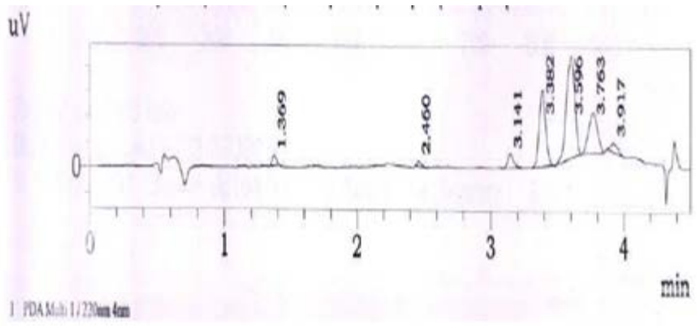

Fig. 9 LCMS analysis: btefore treatment

effluent concentration and at 80 rpm. Above 5% the decolorization activity was reduced.

Significant reduction in COD as well as metals (Cd, Iron, Mn, Zn etc.) was observed in physico chemical analysis after treatment (Data not shown).

After the treatment of same hazardous effluent in designed microbial reactor showed decolorized clear aqueous solution free from kerosene, suspended particles which can also be seen visibly, the same sample was further used for the final chemical analysis, i.e. HPLC and LCMS to trace out the residual chemical contents. Consequently, LCMS and HPLC analysis showed no presence of dyes Scarlet red and Magenta (LCMS ret. time of fragments a, c are 3.95, 3.4 respec-

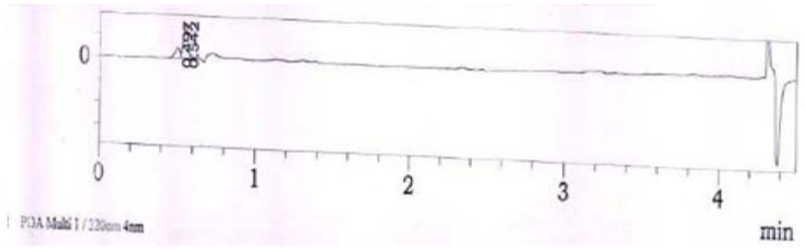

Fig. 10 LCMS analysis: after treatment

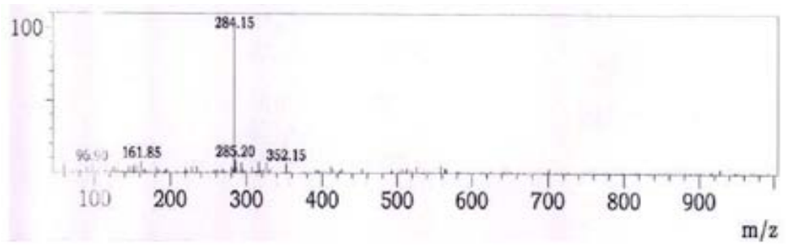

Fig. 11 Mass spectrum

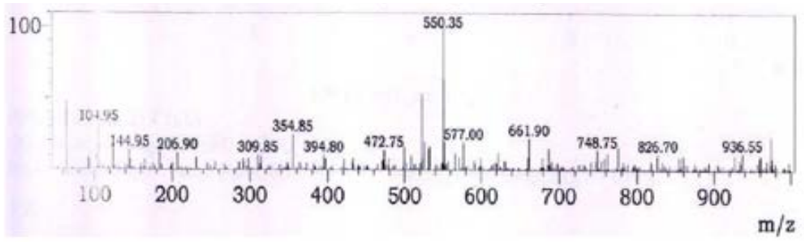

Fig. 12 Mass spectrum

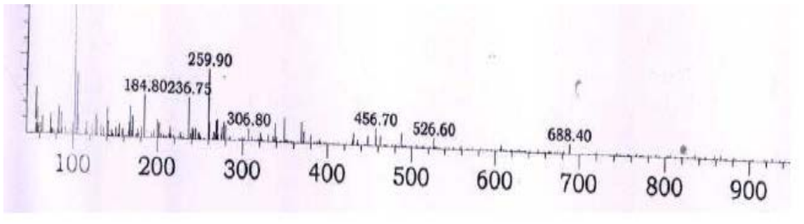

Fig. 13 Mass spectrum

tively). Only some untraceable organic compounds (not ionizing on LCMS) were found in very low and negligible concentrations.

CONCLUSION

The developed bacterial consortium (BM) has the ability to mineralize the dyes present in the effluent.

Experiment aimed for removal of biologically hazardous dye contents from effluent of offset printing industry showed remarkable results. The analysis made before and after the treatment, clearly shows the degradation of residual dye. MBR (mixed bed reactor) has successfully proven the removal of dye from effluent (which can be justifiable by the given data) i.e. the analysis of degraded products showed complete mineralization of dyes Scarlet red and Magenta present in the effluent.

It has been proved that the developed microbial technology i.e. Mixed Bed Reactor is found to be more cost effective and as an acceptable alternative for effluent treatment.

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