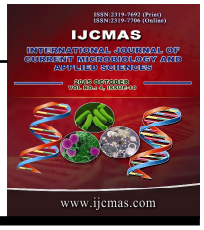
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 10 (2015) pp. 12-17**
http://www.ijcmas.com



Original Research Article

Decolorization Study on Synthetic Colorants by Using Spore Inoculum of *Aspergillus oryzae* JSA-1.

Snehal Agnihotri*

Dr. D.Y.Patil Arts, Commerce and Science College, Pimpri, Pune-411018 India

**Corresponding author*

A B S T R A C T

Physical/chemical treatments of distillery effluent lead to formation of large quantities of sludge as well as simultaneous generation of other hazardous by products or pollutants and not cost effective. The biomethanation process is not efficient in degrading coloring compounds of distillery effluent. The recalcitrant nature of effluent is due to the presence of brown polymers melanoidin, caramel and alkaline degradation products. Biodegradation reduces the load of recalcitrant material from environment and is also cost effective. Development of microbial strains capable of degrading specific pollutants to get a valuable product as a fertilizer for crops is an interesting development in environmental biotechnology. Therefore during last few years attention has been directed towards utilization of specific microbial activity for degrading coloring compounds of distillery effluent. In present study, decolorization study on synthetically prepared colorants by *Aspergillus oryzae JSA-1* was carried out. Media containing melanoidin, caramel and ADP separately in different concentrations were studied for the percent reduction in colorant concentration after fungal growth for twelve days, by reading the absorbance at 330 nm, 283 nm and 264 nm respectively (optimum wave lengths for melanoidin, caramel and ADP) before and after fungal treatment. It indicated that the percent removal of colorants by treatment with *Aspergillus oryzae JSA-1* decreased on increasing concentration of colorants.

Keywords

Synthetic colorant, melanoidin, caramel, ADP, decolorization, absorbance

Introduction

In sugar manufacturing, during separation of sugar from non sugar, sugar itself is subjected to number of shocks of alkali, acid and heat. These shocks are actually responsible for formation of sugar decomposition color (Kort, 1979). The coloring matter produced during sugar manufacturing is initially concentrated into molasses and further carried forward into spent wash and then to effluent collected after its biomethanation treatment (Dhamankar, 2001). There are three major colorants formed under high temperature, such as melanoidin, caramel and alkaline degradation products. Different workers (Murata *et al.*, 1992; Watanabe *et al.*, 1982; Aoshima *et al.*, 1985; Ohmomo *et al.*, 1988; Roland, 1989) carried out biological

decolorization by the application of microbial cultures for model melanoidin and distillery waste water containing melanoidin under laboratory conditions because melanoidin possesses higher potential for the production of dark brown color of the effluent even though it is present in trace amounts. Guimaraes *et al.* (1999) have studied decolorization and degradation of colorants of sugar refinery such as melanoidin, caramel, ADP and polyphenols. The fungal culture was able to decolorize melanoidin, caramel, ADP and polyphenols by 74%, 87%, 80% and 72% respectively. *Aspergillus niger UM 2* was shown to have strong potential to decolorize biomethanated effluent by reducing melanoidin, caramel, ADP and polyphenol contents and reduction in levels of other effluent parameters like COD, TOC, sulphate, phosphate, chloride (Patil *et al.,* 2001; Dhamankar and Patil, 2004). The present study deals with the synthetic colorants which are responsible for imparting color to the effluent. Modes of interaction of each colorant separately can explore the mechanism of color removal by the fungal culture. Earlier reports (Kort, 1979; Dhamankar, 2001; Murata *et al.,* 1992; Watanabe *et al.,* 1982; Aoshima *et al.,* 1985; Ohmomo *et al.,* 1988; Roland, 1989) on microbial decolorization of spent wash suggest that microbial decolorization technology is difficult to develop at industrial scale due to lack of proper microbial strain, which could decolorize effluent efficiently at less dilution.

The process optimization studies are needed for making any biological process viable at pilot or industrial scale. In present study different process parameters were standardized for decolorization of melanoidin, caramel and ADP which were synthetically prepared in the laboratory, in a series of batch experiments by using the fungal isolate, '*Aspergillus oryzae JSA-1',* under shake flask condition (150 rpm).

Materials and methods

Preparation of synthetic colorants

Melanoidin: Mixture of glucose 1M, glycine 1M and sodium carbonate 0.5 M was autoclaved for two hours at 121°C and dialyzed against distilled water. The dark brown colored portion remained after complete dialysis was used as melanoidin (Ohmomo *et al.,* 1985).

Caramel: Mixture of Sucrose (180 gm) and deionized water (20 ml) was heated at 105°C for 66 hours (Cookson *et al.,* 1970).

ADP*: Glucose solution (20 g/lit, pH 9.5) was refluxed for five hours. pH was constantly maintained at 9.5 with NaOH throughout refluxing (Annet, 1957).

* Alkaline degradation products

Decolorization study on synthetic colorants by using spore inoculum of *Aspergillus oryzae JSA-1*

All experiments were carried out in 250 ml Erlenmeyer's flasks, each containing aliquots of the medium (GPM) containing glycerol, 5%; peptone, 0.5%; KH_2PO_4, 0.1%; $MgSO_4.7H_2O$, 0.05%, at pH 6. Synthetically prepared melanoidin, caramel and ADP in the concentrations of 50, 75, 100 and 200 mg/kg were added separately to aliquots of the media. All flasks were autoclaved and inoculated with culture and incubated on rotary shaker (150 rpm) at 30°C for 12 days. The optical densities of the culture filtrates were found out at zero

day, 4 days, 8 days and 12 days incubation, by reading the absorbance at 330 nm, 283 nm and 264 nm (Shimadzu UV-240) for melanoidin, caramel and ADP respectively (Table 1). Percent reduction in concentration of each colorant after fungal treatment with filtration was found out by using standard graph of each colorant. All flasks were analyzed for dry weight mycelia and final pH of the culture filtrates. All reagents used were of analytical grade and all the experiments were done in triplicates.

Decolorization study on colorants of biomethanated distillery effluent by using spore inoculum of *Aspergillus oryzae JSA-1*

In 250 ml Erlenmeyer's flasks, aliquots of the basal medium (GPM) with 30% BME (biomethanated effluent) were taken, autoclaved and inoculated with culture. The flasks were incubated on rotary shaker (150 rpm) at 30°C for 12 days. The optical densities of the culture filtrates were found out after 4 days, 8 days and 12 days incubation, by recording the absorbance at 330 nm, 283 nm and 264 nm for melanoidin, caramel and ADP respectively (Table 2). Percent reduction in concentration of each colorant after fungal treatment was found out by using standard graph of each colorant Percent decolorization was determined by reading the absorbance of culture filtrate at 475 nm (absorption maxima of filtered BME) before and after fungal treatment.

Results and discussion

Decolorization study on synthetic colorants by using spore inoculum of *Aspergillus oryzae JSA-1*.

The percent decolorization of synthetic colorants obtained by using spore inoculum of *Aspergillus oryzae JSA-1* are shown in table 1.

When the culture was grown for 12 days in sterile base media containing 50 mg%, 75 mg%, 100 mg% and 200 mg% melanoidin, caramel and ADP separately, it was seen that percent reduction of melanoidin was 47.98 ± 1.23% when the concentration of melanoidin present in basal medium was 50 mg%. However it was lowered down to 39.70 ± 1.12%, 36.80 ± 0.09% and 30.20 ± 0.51% when concentration of melanoidin in the basal medium was increased up to 75, 100 and 200 mg% respectively. Similarly in case of caramel and ADP the percent reduction was 48.09 ± 1.32% and 45.08 ± 1.21% respectively when the concentration of colorants were 50 mg% and found to be lowered down to 28.3 ± 0.05% and 25.2 ± 0.16% respectively by increasing colorant concentration up to 200 mg%. The results indicated that the percent removal of colorants by the isolate decreased on increasing concentration of colorants (Fig. 1).

Decolorization study on colorants of biomethanated distillery effluent by using spore inoculum of *Aspergillus oryzae JSA-1*

When the medium containing biomethanated distillery effluent was inoculated with spores of *Aspergillus oryzae JSA-1* for 12 days, and studied for the percent reduction of colorants, it was found that percent reduction of melanoidin was 39.07 ± 1.23%. Caramel and ADP were found to be decolorized up to 26.47 ± 1.08% and 27.35 ± 2.12% respectively. This might be due to the fact that, the concentration of these colorants in distillery effluent was higher (270, 1700, 1060 mg% of melanoidin, caramel and ADP respectively) than the concentration of synthetic colorant tested (50, 75, 100 and 200 mg%). However the percent reduction of total color of effluent medium (O.D. at 475 nm) was found to be 77.7 ± 3.16% in 12 days incubation (Table 2).

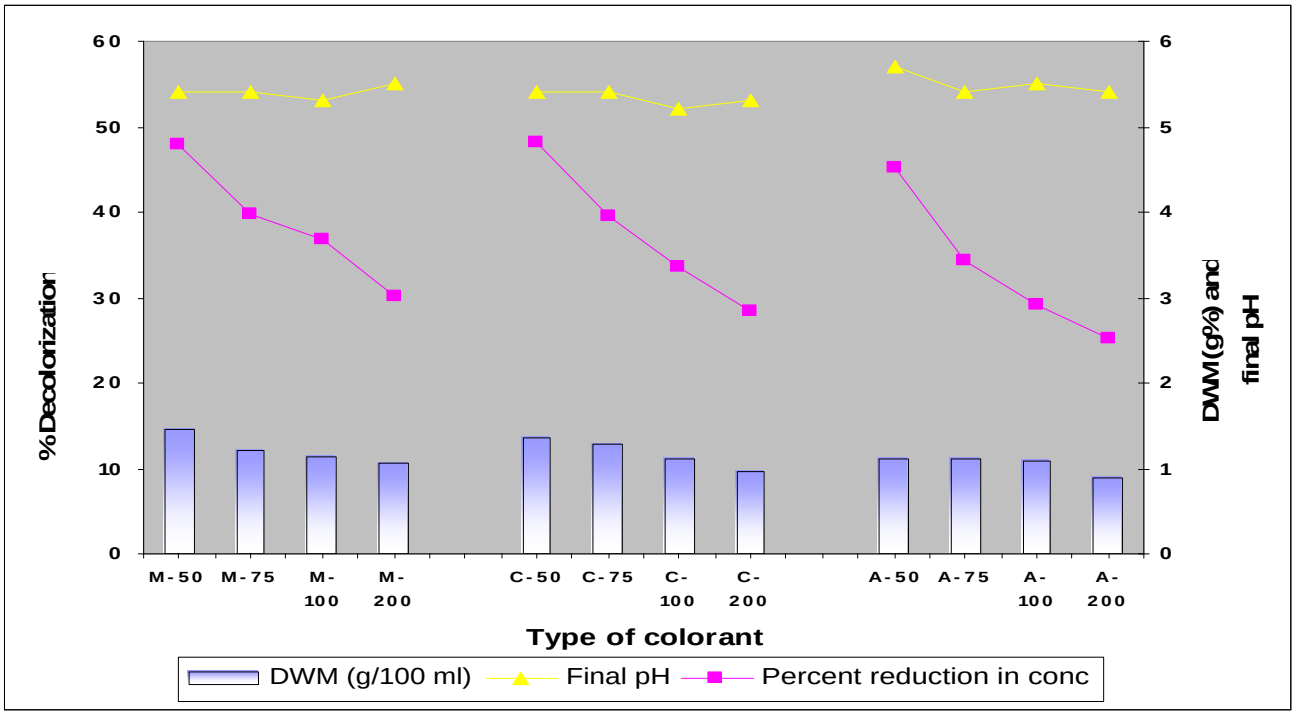
Table.1 Percent reduction in concentration of synthetic colorants by using spore inoculum of *Aspergillus oryzae JSA-1*

Type of Colorant	Concentration of colorant (mg %) in the medium				%Reduction in concentration of colorant (12^{th} day)	DWM in g/100 ml medium (12^{th} day)	Final pH (12^{th} day)
	0 Day	4th Day	8th day	12th Day			
Melanoidin	50	38.47	29.4	26.01	47.98 ± 1.23	1.445	5.4
	75	59.85	48.4	45.2	39.70 ±1.12	1.214	5.4
	100	80.2	68.7	63.2	36.80 ± 0.09	1.134	5.3
	200	168.6	153.4	139.6	30.20 ± 0.51	1.066	5.5
Caramel	50	34.05	31.8	25.95	48.09 ± 1.32	1.104	5.7
	75	48.2	47.1	45.3	39.60 ± 1.16	1.123	5.4
	100	86.1	73.4	66.5	33.50 ± 0.08	1.098	5.5
	200	163.2	149.8	143.4	28.30 ± 0.05	0.897	5.4
ADP	50	37.05	32.9	27.46	45.08 ± 1.21	1.104	5.7
	75	55.6	53.2	49.35	34.20 ± 1.32	1.123	5.4
	100	89.8	83.2	70.4	29.10 ± 0.07	1.098	5.5
	200	173.4	151.8	149.6	25.20 ± 0.16	0.897	5.4

Table.2 Percent reduction in concentration of colorants in biomethanated distillery effluent by *Aspergillus oryzae JSA-1*

Parameter	**0 Day**	**4 Days**	**8 Days**	**12 Days**	**% Reduction**
Color (O.D. 475)	5.85	3.24	2.02	1.3	77.7 ± 3.16
Melanoidin (mg %)	270	250	190	164.5	39.07 ± 1.23
Caramel (mg %)	1700	1530	1350	1250	26.47 ± 1.08
ADP (mg %)	1060	850	800	770	27.35 ± 2.12

Fig.1 Percent removal of synthetically prepared colorants on increasing concentration of colorants in the medium by using spore inoculum of *Aspergillus oryzae JSA-1* culture (12 days incubation) under shake flask conditions



Abbreviations used in the figure

M-50: Melanoidin 50 mg%; **M-75**: Melanoidin 75 mg%; **M-100**: Melanoidin 100 mg%; **M-200**: Melanoidin 200 mg%; **C-50**: Caramel 50 mg%; **C-75**: Caramel 75 mg%; **C-100**: Caramel 100 mg%; **C-200**: Caramel 200 mg%; **A-50**: ADP 50 mg%; **A-75**: ADP 75 mg%; **A-100**: ADP 100 mg%; **A-200**: ADP 200 mg%.

The results in this study showed that as the concentration of colorant in the medium or effluent increases the percent decolorization decreases. This might be due to availability of the sites responsible for adsorption of color on the cell walls of the fungal culture. Once the sites are saturated with adsorbed pigment there are no vacant sites for adsorption of color. The other components present in the effluent might also be interfering with the process of decolorization of the effluent.

The present study on decolorization of synthetic colorants by spore inoculum of *Aspergillus oryzae JSA-1* (12 days incubation) showed that the percent decolorization of each colorant was found to be decreasing with increasing concentration of the colorant.

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