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Decolorization of Distillery Wastewater by UV irradiated spores of *Aspergillus fumigatus*

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Abstract
An efficient microbial technique for decolorizing distillery wastewater was achieved using irradiated spores of Aspergillus fumigatus. The fungus was isolated from soil samples taken from the local distillery processing unit. By using the irradiated spores, wastewater decolorization increased by 14.3% as compared to that of the control treatment (68.8% vs. 54.5%). In the presence of sodium nitrate and maltose, as optimum sources of nitrogen and carbon, in the microbe's growth medium, the decolorization rose to 70%.

Keywords: *Aspergillus fumigatus, decolorization, distillery wastewater, UV irradiation*

Introduction

Due to its high carbon content, low cost and availability, molasses is one of the most important raw materials used in the commercial production of ethanol [1]. In an ethanol distillery, depending on the process employed, between 10-15 liters of wastewater are produced for every liter of alcohol produced.

Distillery wastewater is a highly colored effluent with the potential to cause eutrophication of waterways due to its high-pollutant load and high COD of the order of 90,000 mgl^{-1}[2]. The colored nature of distillery wastewater is due to the presence of natural polymers called melanoidins, formed by the Maillard amino carbonyl reaction [3]. Due to the recalcitrant nature of melanoidins, conventional wastewater treatment processes are unable to remove the color from distillery wastewater. The latter then has the potential to block out light from contaminated waterways thus preventing oxygenation [1]. In addition to being a water pollutant, distillery wastewater also causes manganese deficiency when disposed of in soil, resulting in a loss of soil fertility [4].

In order to remove color from wastewater of distilleries, several species of terrestrial fungi have been tried [1, 5, 6].

In this study we report the effect of UV irradiation, screening of carbon and nitrogen sources on *Aspergilles fumigatus* fungi isolated from soil samples of Bidestan Distillery and Food Products, Qazvin, Iran.

This innovative approach towards strain improvement by mutation was applied to test the feasibility of performing a large scale decolorization process. With this aim, a population of spores of *A. fumigatus* was

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subjected to electromagnetic radiation. Empirically, an optimal irradiation time was determined which killed a large proportion of the population while allowing a percentage of genetically modified individuals to survive. Survivors were then isolated and screened for the improved decolorizing characteristics. The application of this method was shown to be effective for decolorization of distillery wastewater.

Materials and Methods

Media and culture conditions

Aspergillus fumigatus U_{B2} isolated from soil samples collected from Bidestan Distillery Ltd., was grown on PDA slant for seven days. Culture media (100 ml) was prepared in a 500-ml flask consisted of (gl^{-1}); glucose, 70; KH_2PO_4, 1; KCl, 0.5; $NaNO_3$, 2; $MgSO_4.7H_2O$, 0.5; $FeSO_4$, 0.001 and pH was adjusted to 4.5 and inoculated with 8×10^7 spores per liter. In order to study the decolorization activity of the UV treated spores, the decolorization medium was prepared as follows (gl^{-1}): glucose, 15; $NaNO_3$, 1.5; KH_2PO_4, 1; cell dry weight, 2.5 and pH was adjusted to 5. Media were cultured at 30°C on a rotary shaker at 160 rpm. The decolorization experiments were carried out in two sets, each at different times with two replications.

Decolorization assay

Samples were drawn every 24 h and centrifuged at 8000 rpm for 10 min. The supernatant was used for determination of percentage decolorization. Percentage decolorizetion in each sample was measured as a decrease in optical density at 475 nm using a PYE UNICAM spectrophotometer. The percentage decolorization was expressed as the degree of decrease in absorption at 475 nm over the initial absorption at the same wavelength according to the formula [2]:

$$Percentage\ declolorization = \frac{Initialabsorbance\ (475nm)}{Initialabsorbance\ (475nm)} * 100$$

Pretreatment of distillery wastewater

Distillery wastewater was first anaerobically digested and aerobically treated in advance in Bidestan distillery wastewater plant. Pretreated distillery wastewater (without dilution) was used in the present research.

UV irradiation

Isolated *A. fumigatus* U_{B2} were irradiated according to the method of Champness [7]. 10 ml of spore suspension containing $5x10^4$ spores/ml in a sterile Petri dish were UV irradiated for different times keeping the Petri dishes at a distance of 5 cm from UV source. The spores were UV irradiated for a period of 40, 60, 80 and 100 sec. After UV irradiation, they were cultivated on the surface of PDA media in Petri dishes and left at 30°C for colony formation. The colonies were checked for best results and compared with the intact (i.e. not irradiated) spores.

Statistical Analysis of Data:

To compare the means of experimental results, analysis of variance (ANOVA) was employed [8]. Analysis of variance (ANOVA) is a statistical approach to estimate the variances about the population means (within group variances and between group variances estimates). LSD (Fisher's Least Significant Difference) is a test procedure developed to compare the results after conducting ANOVA.

To provide a sound basis for future studies, the effect of different treatments (i.e. UV irradiation dose, nitrogen and carbon sources) on decolorization, all experiments were repeated. This repetition was the reason for using a randomized complete block design to separate the differences due to the batches, treatments and experimental errors.

Results and Discussion

A. fumigatus U_{B2} was selected after screening

twenty-one known and isolated micro-organisms for its highest percentage decolorizetion of pretreated distillery wastewater during previous studies [9]. The organism decreased the color of diluted (25% v/v) pretreated distillery wastewater by 80%.

In order to improve the percentage decolorization of the microorganism, it was UV irradiated at different times and its percentage decolorization was compared with that of untreated spores.

Effect of UV irradiation

The spores were UV irradiated for a period of 40, 60, 80 and 100 sec. Subsequently, they were cultivated and their percentage decolorization values (see definition above) compared. The result of maximum percentage decolorization by irradiated and intact spores are shown in Table 1 [7].

From the results shown in Table 1, it is apparent that UV irradiation had an effect on the percentage decolorization achieved by *A. fumigatus* U_{B2}. The intact microorganism had a mean percent decolorization of 54.53 while those irradiated had a mean percent decolorization of 43.85, 68.83, 44.12, and 47.10 respectively at 48 h of cultivation. Considering the different times of irradiation, the 60 sec irradiation increased the mean percentage decolorization by 14.3, compared to the control spores. The 60 sec UV irradiated microorganism is referred to as *A. fumigatus* $U_{B2}60$ in the further discussion. Table 2 shows the analysis of variance for the investigation of effect of UV irradiation on percentage decolorization of pretreated distillery wastewater. It is apparent from the variance ratio of treatment mean square to the errors mean square that the calculated value of $F_0 = 1326.59$ is much more than $F_{statistics}$ (3.11) at $\alpha = 0.05$. Comparing the mean in Table 1 it is suggested that the UV irradiation has changed the sequence of base pairs in the DNA of the microorganism. After analysis of variance the least square difference has been calculated and the value obtained was 1.23. The mean values shown in Table 1 indicate that there is a difference between all the means considering the LSD value and that the mean value of percentage decolorization for 60 sec irradiation shows a larger effect than others in improving the percentage decolorization.

Table 1. Maximum percentage decolorization before and after UV irradiation of *A. fumigatus* U_{B2} using pretreated distillery wastewater

Irradiation time (Sec)	Batch 1		Batch 2		Mean percentage decolorization
	Trial 1 %	Trial 2 %	Trial 1 %	Trial 2 %	
Without irradiation	54.80	54.44	54.09	54.80	54.53
40 Sec	44.76	43.94	43.02	43.68	43.85
60 Sec	68.80	69.65	67.68	69.19	68.83
80 Sec	44.80	43.70	44.25	43.75	44.12
100 Sec	47.45	46.50	47.45	47.00	47.10

Table 2. Analysis of variance for the data obtained to test the effect of UV irradiation of *A. fumigatus* $U_{B2}60$

Sources of variation	SS	df	MS	F
Treatment	1766.49	4	441.6225	1326.59
Batch	0.77237	1	0.77237	2.320
Residual	4.66123	14	0.3329	
Total	1771.9237	19		

LSD = 1.23

Effect of nitrogen source

In order to study the effect of different nitrogen sources for future studies on percentage decolorization of *A. fumigatus* $U_{B2}60$ a number of nitrogen sources namely $NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$, NH_4Cl, yeast extract, and urea were incorporated in decolorizing media independently. The results are shown in Table 3. From the mean percentage decolorization shown in Table 3 it is apparent that each nitrogen source had a different percentage decolorization and that $NaNO_3$ had the maximum percentage decolorization of 68.77 at 48 h of cultivation.

The result of analysis of variance is listed in Table 4. The calculated F_0 value (48.44) is greater than the $F_{statistic}$ (2.60) at probability level of $\alpha = 0.05$ and hence the nitrogen source has an effect on the percentage decolorization. The calculated LSD value for this experiment is 2.78. From the mean percentage decolorization, the value of LSD and the higher difference between the mean percentage decolorization of other nitrogen sources, it is concluded that $NaNO_3$ is the best nitrogen source among those studied for this fungi; it appears highly promising for future studies.

Effect of carbon source

The effect of different carbon sources namely: glucose, fructose, sucrose, maltose, mannose, ribose, and also the absence of carbon source on percentage decolorization of *fumigatus* $U_{B2}60$ were studied by incorporating each carbon source in the decolorizing medium independently. From the observation of the result in Table 5 it is apparent that maltose and glucose had higher mean percentage decolorization values of 70.60 and 68.66 respectively, than the other carbon sources.

The analysis of variance of the results (Table 6) also indicates the effect of different carbon sources on percentage decolorization. The calculated F value (67.18) also shows the influence of different carbon sources on percentage decolorization, which is higher than the $F_{statistic}$ (2.60) at $\alpha = 0.05$.

The least significance difference calculated is 1.37 and it is apparent that there is a significant difference between the mean percentage decolorization of some carbon sources but this difference is higher among maltose and glucose (70.60 and 68.66 respectively) compared to the rest of carbon sources. The difference of percentage decolorization for maltose and glucose as carbon source is small (1.94) but the value is higher than the LSD value and maltose is selected as a best carbon source for further studies. Maltose has also been reported [10] for maximum percentage decolorization after screening a number of carbon sources for *A. fumigatus* with similar morphological characteristics and the same purpose. Authors during the optimization and scale up have also checked and confirmed the superiority of maltose to glucose.

Table 3. Effect of nitrogen source on percentage decolorization of DW of *A. fumigatus* $U_{B2}60$

Nitrogen sources	Batch 1		Batch 2		Mean percentage decolorization
	Trial 1 %	Trial 2 %	Trial 1 %	Trial 2 %	
$NaNO_3$	69.40	70.27	68.3	67.10	68.77
NH_4NO_3	55.96	54.76	52.69	56.97	55.09
$(NH_4)_2SO_4$	59.83	57.35	55.96	57.55	57.67
NH_4Cl	56.67	55.80	57.55	56.12	56.53
Peptone	58.92	56.70	56.90	56.70	57.30
Yeast extract	64.10	62.18	60.68	61.98	62.23
Urea	58.45	59.30	61.84	57.61	59.30

Table 4. Analysis of variance for effect of nitrogen sources on percentage decolorization of DW by *A. fumigatus* $U_{B2}60$

Sources of variation	SS	df	MS	F
Treatment	516.2354	6	86.04	48.44
Batch	5.342	1	5.342	
Residual	35.522	20	1.776	
Total		27		

LSD = 2.78

Table 5. Effect of carbon source on decolorization efficiency of *A. fumigatus* $U_{B2}60$

Carbon sources	Batch 1		Batch 2		Mean percentage decolorization
	Trial 1 %	Trial 2 %	Trial 1 %	Trial 2 %	
Glucose	68.80	69.05	67.65	69.15	68.66
Fructose	59.78	62.06	63.29	61.19	61.58
Sucrose	62.38	55.17	60.44	59.02	59.25
Maltose	71.51	68.70	71.86	70.34	70.60
Mannose	56.73	54.15	53.65	51.60	54.03
Ribose	54.54	53.17	55.08	53.47	54.06
Without carbon source	52.40	51.22	54.44	51.96	52.5

Table 6. Analysis of variance for the effect of carbon source on decolorization efficiency of *A. fumigatus* $U_{B2}60$

Sources of variation	SS	df	MS	F
Treatment	1269.7885	6	211.63	67.18
Batch	0.4325	1	0.4325	
Residual	63.0206	20	3.151	
Total	1333.2416	27		

LSD = 1.37

Conclusion

UV irradiation of *Aspergillus fumigatus* spores resulted in their improved ability to decolorize distillery waste water. The percentage decolorization rose by 14.3. Among the nitrogen and carbon sources studied, $NaNO_3$ and maltose had the highest decolorizing efficiency and should be considered in the future studies with *A. fumigatus* $U_{B2}60$. We believe that application of our strain U_{B2} in the treatment of distillery wastewater could be more practical than that of C*oriolus* species no. 20 [11], *Aspergillus fumigatus* sp. G-2-6 [10], Rhizoctonia sp. D-90 [12] due to the high decolorization activity and COD removal [13].

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