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# Biodegradation of basic fuschin dye by *Saccharomyces cerevisiae* isolated from "kunun-zaki" (A locally fermented beverage in Nigeria)

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## ABSTRACT

Basic fuschin dye belongs to the most important group of synthetic colorants and is used extensively in the textile industries and clinical laboratories. Wastewaters from these industries and laboratories washed down into flowing streams and rivers posed serious health hazard to the receiving communities. In this study, *Saccharomyces cerevisiae* isolated from a commercially prepared kunun-zaki was used to degrade 20mg basic fuschin dye for a period of 12 days under aerobic condition in 250, 500 and 750mL mineral salt media. The degree of decolourization of basic fuschin was determined using UV-visible spectrophotometer with absorbance of 620nm. At the end of twelve days 68%, 67% and 69% basic fuschin decolourization were recorded in 250mL, 500mL and 750mL concentration, respectively. The results suggest the potential of *Saccharomyces cerevisiae* for the treatment of wastewater containing basic fuschin. © 2014 Trade Science Inc. - INDIA

## KEYWORDS

Biodegradation;
Basic fuschin dye;
Kunun-zaki;
*Saccharomyces cerevisia.*

## INTRODUCTION

Basic fuchsin is a triphenylmethane dye made up of some chemically related dyes such as Basic red 9 (Magenta 0), Magenta1 (Rosaniline), Magenta11, and Magenta111 (New fuchsin). The dye was among the first to be produced beginning in 1850s[1]. Maganta is used in hair dye, as colourant in artist paints, as cosmetics products not intended to come in contact with mucous membranes, to satin animal and vegetable fibres[2]. Wastewater containing this dye from textile industries is difficult to treat using conventional method because it is stable to light and oxidizing agents, and are resistant to aerobic digestion[3,4]. The general population can be exposed to this dye through the use and production of the dye. Occupational exposure can also occur during the use and production of basic fuchsin as a dye intermediate and when dyeing textile fibres, fabrics, and paper product[5]. Rehn[1] was the first to report the appearance of bladder tumours in three of 45 workers involved in the manufacture of basic dyes at Germany. Conventional biological wastewater treatment systems are often incapable of effectively removing dyes from wastewater, resulting in its dispersal into the environment.

The microbial decolourization of dyes has been of considerable interest due to their inexpensive and ecofriendly nature as well as producing a less amount of sludge[6]. Presently, an extensive research has been

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carried out to discover the potent microbial biomass which is as cheap as possible for the removal of dyes from large volumes of polluted water[7]. Most of the processes used in the treatment dye wastewater are chemical processes which are costly, produce large amount of sludge, and are less efficient. Biological processes are getting more attention since it is cost effective, environmentally friendly, and does not produce large quantities of sludge.

Many researchers have reported the biodecolourization of various dyes using organisms obtained from different extracts. For example, Yatome*et al.*[8] reported the decolorization of crystal violet by two Actinomycetes species such as *Nocardiacorallina*and*Nocardiagloberula*and found out that the dyes were completely decolorized in 24 hours. Yatome *et al.*[8] also reported the decolourization of four triphenylmethane dyes such as basic fuchsin, methyl violet, crystal violet, and victoria blue by bacteria, *Pseudomonas pseudomallei*and found out that methyl violet and crystal violet were appreciably decolorized while basic fuchsin and victoria blue were not decolorized under experimental condition. Kwasniewska[9] showed that oxidative yeast such as *Rhodotorulasp*and *Rhodotorularubra*were capable of degrading crystal violet in liquid broth. The biodecolourization of basic fuchsin by yeast extracted from *kunun-zaki*was not documented.

Chemical processes are extensively used for the degradation of dyes by different dye manufacturing and dyeing industries[10]. However, these processes are not safe to use and are recalcitrants. Biological processes are encouraged because they are safer and environmentally friendly. Therefore, the aim of the present investigation was to study the biodegradation of basic fuschin dye by *Saccharomyces cerevisiae*isolated from kununzaki (a locally fermented beverage in Nigeria made from Sorghum).

## MATERIALS AND METHODS

### Sample collection and preservation

The basic fuschin dye used in this study was obtained from the Department of Biological Science, Federal University of Technology Minna, Nigeria. A commercially prepared *kunun-zaki* was bought from the market and preserved in the refrigerator. Other chemicals used in the analysis were of analytical grade.

### Isolation and identification of yeast isolates

Yeast was isolated from kunun-zaki using pour plate technique. Serially diluted sample of kununzaki was plated on sabouraud dextrose agar (SDA) and incubated at ambient laboratory temperature ($28^0$C for 48 hours). Colonies that appeared on the plates were further subcultured on fresh SDA to obtained pure cultures. The pure cultures were characterized according to the methods of[11] based on their morphological characteristics, which show white colonies and the cell shape,which was gram positive and ellipsoidal in shape microscopically. The isolated yeast colonies were maintained on sabouraud dextrose agar (SDA) slants and stored in the refrigerator for further identification and characterization. Gram's reaction, sugar fermentation and nitrate reduction tests were carried out on the yeast isolates.

### Screening of yeast isolates for potential to degrade basic fuchsin dye

The broth cultures of yeast (*Saccharomyces cerevisae*) were used to investigate their abilities to degrade basic fuschin dye. They were prepared in nutrient broth and incubated at ambient laboratory temperature $28 \pm 2^0$C for 48hours[12].

### Decolourization of basic fuschin dye by yeast

The decolourizing medium (mineral salt) was prepared using 1Litre of distilled water according to Deivasgamani and Das[13] with a little modification. The mineral salt medium was composed of yeast extract-2.0g$L^{-1}$, NaCl-0.5g$L^{-1}$, $NH_4Cl$-1.0g$L^{-1}$, $MgSO_4$- 3g$L^{-1}$, $(NH_4)_2SO_4$ 0.3g$L^{-1}$, $KH_2PO_4$- 0.5g$L^{-1}$, $NaHCO_3$- 1.0g$L^{-1}$, $CaCl_2.6H_2O$- 0.2g$L^{-1}$, $Na_2B_2O_7.10H_2O$-0.2g$L^{-1}$, $MnCl_2.4H_2O$-0.1g$L^{-1}$, $ZnSO_4.7H_2O$-0.1g$L^{-1}$ and $CuSO_4.5H_2O$. 20mg of basic fuschin dye was added to 250mL, 500mL and 750mL of the mineral salt media, respectively. The pH was adjusted to 6.5 with 0.1N HCl and 0.1N NaOH using pH meter (JENWAY, Model 3020). The media containing basic fuschin dye were autoclaved

at 121$^0$C for 15 minutes. 2mL of 24hours cell cultured in nutrient broth media were inoculated into eight (8) conical flasks containing 20mL of the growth media and basic fuschin dye in 50mL Erlenmeyer flask. All experiments were performed in duplicate. The flasks were plugged with sterile cotton wool and incubated at ambient laboratory temperature. Aerobic condition was provided by shaking the flasks as described by Deivasgamani and Das[13] throughout the duration of the experiment. The initial absorbance of basic fuschin dye was taken at 630nm using UV-spectrophotometer (M$^R$ 752 UV – spectrophotometer, Model YM1208PTS1) after centrifugation at 1200rpm for 15 minutes. The experiment lasted for 12 days and absorbance was taken at three days interval along side the control, which was necessary in order to monitor the decolourization of the dye by the yeast isolate.

## Decolourization assay

The decolourization was measured using UV-spectrophotometer at 630nm. The percentage decolourization was calculated from the following equation as shown below according to Saranraj *et al.*,[14].

$$\% \text{ Decolourization} = \frac{Ao - At}{Ao} \times 100$$

## RESULTS AND DISCUSSIONS

TABLE 1 shows the percentage degradation of basic fuschin dye at 20mg/250ml concentration. The dye was degraded effectively on the 12$^{th}$ day with 68.2%. On the 3$^{rd}$ day 43.4% degradation was recorded while between the 6$^{th}$ and 9$^{th}$ day it was found to be 50.39% and 67.79%, respectively. TABLE 2 shows the percentage degradation of basic fuschin dye at 20mg/500ml concentration. The dye was degraded effectively on the 12$^{th}$ day with 67.38%. On the 3$^{rd}$ day 40.82%degradation was recorded while between the 6$^{th}$ and 9$^{th}$ day it was found to be 45.15% and 61.40%, respectively. TABLE 3 shows the percentage degradation of basic fuschin dye at 20mg/750ml concentration. The dye was degraded effectively on the 12$^{th}$ day with 69.02%. On the 3$^{rd}$ day 54.00%degradation was recorded while between the 6$^{th}$ and 9$^{th}$ day it was found to be 61.92% and 68.92%, respectively.

**TABLE 1: Degradation of basic fuschin dye by *Saccharomyces cerevisiae* in 20mg/250mL**

| Incubation Period (days) | Concentration of Dye (mg) | | % Degradation |
|---|---|---|---|
| | Control | Treatment with *SC* | |
| 3 | 16.28 | 11.32±2.13 | 43.40 |
| 6 | 14.84 | 9.92±1.31 | 50.39 |
| 9 | 11.96 | 6.44±0.18 | 67.79 |
| 12 | 8.98 | 6.34±0.09 | 68.20 |

**SC= *Saccharomyces cerevisiae***

**TABLE 2 : Degradation of basic fuschin dye by *Saccharomyces cerevisiae* in 20mg/500mL**

| Incubation Period (days) | Concentration of Dye (mg) | | % Degradation |
|---|---|---|---|
| | Control | Treatment with *SC* | |
| 3 | 16.24 | 11.84±0.58 | 40.82 |
| 6 | 16.32 | 10.97±0.12 | 45.15 |
| 9 | 15.66 | 7.72±0.99 | 61.40 |
| 12 | 14.80 | 6.53±0.64 | 67.38 |

**SC= *Saccharomyces cerevisiae***

**TABLE 3: Degradation of basic fuschin dye by *Saccharomyces cerevisiae* in 20mg/750mL**

| Incubation Period (days) | Concentration of Dye (mg) | | % Degradation |
|---|---|---|---|
| | Control | Treatment with *SC* | |
| 3 | 13.44 | 9.02±1.08 | 54.00 |
| 6 | 13.40 | 7.62±0.90 | 61.92 |
| 9 | 10.48 | 6.22±0.96 | 68.92 |
| 12 | 6.94 | 6.20±0.99 | 69.02 |

**SC= *Saccharomyces cerevisiae***

The *Saccharomyces cerevisiae* decolourized basic fuschin dye in the range of 43.40 to 68.20%, 40.82 to 67.38% and 54.00 to 69.02% between 3 to 12 days incubation period under aerobic condition. Similar results were reported on biodegradation of azo dyes by yeast (*Issatchenkiaoccidentalis*), where maximum decolourization was observed under aerobic conditions[15]. They further reported that under anoxic condition decolourization was lesser due to absence of metabolic activities.

The decolourization in 20mg/250mL concentration increased with increased incubation period from 3 to 12 days. The concentration of 20mg/500mL showed decolourization increasing as the incubation period increased while in 20mg/750mL, the decolourization also increased with increase in in-

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cubation period up to 12days. Similar results were reported by Daivasigamani and Das[13] in their work on biodecolourization of basic violet-3 by *Candida krusei* isolated from textile wastewater. They reported maximum decolourizations of 74% and 100% in the media supplemented with sucrose and sugarcane bagase extract within 24 hours, respectively.

The results showed that *Saccharomyces cerevisiae* is capable of degrading basic fuschin dye and utilizing the dye as sole carbon and energy source for the cell growth within the incubation period considered. Saranraj*et al.*[14] reported in a similar study, biodecolourization of textile dye effluent by *Aspergillus species*, *Fusariumoxysporum*, *Penicilliumchrysogenum*, *Mucor species* and *Trichodermaviridei*.

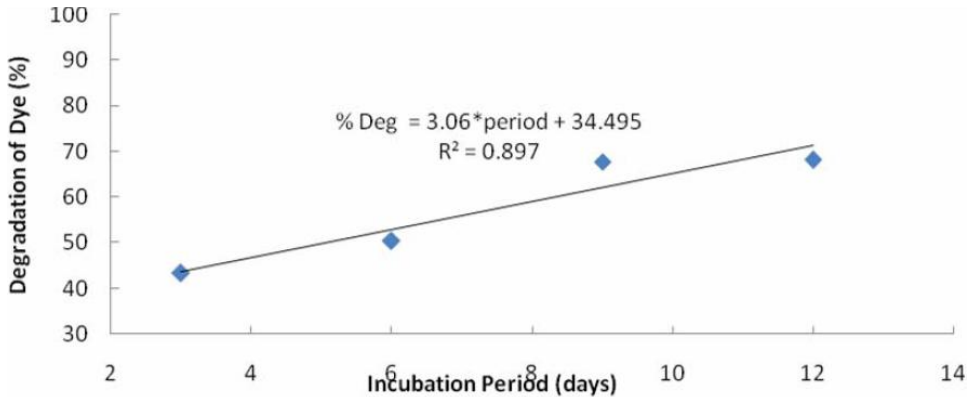


**Figure 1 : Effect of incubation period on degradation of 20mg/250mL basic fuschin dye by *Saccharomyces cerevisiae***

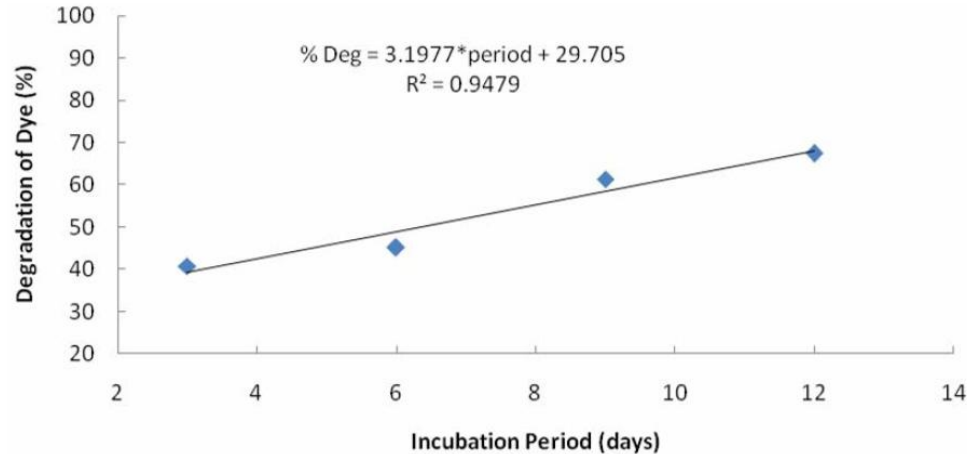


**Figure 2 : Effect of incubation period on degradation of 20mg/500mL basic fuschin dye by *Saccharomyces cerevisiae***

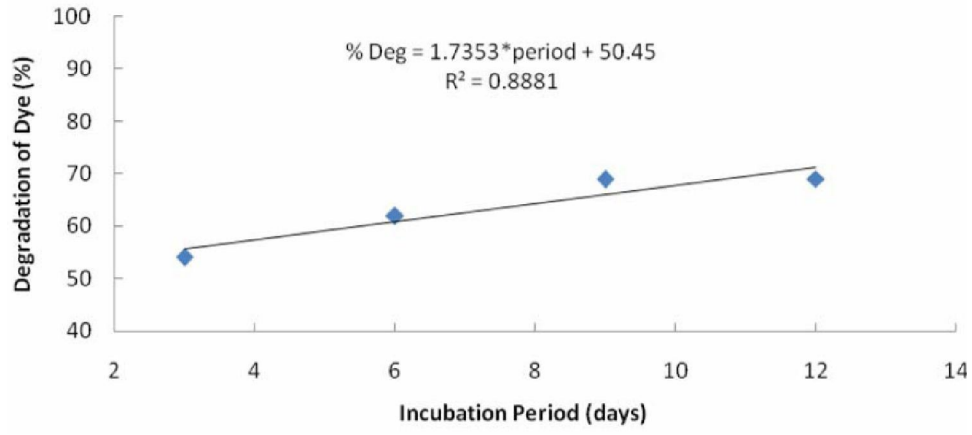


**Figure 3 : Effect of incubation period on degradation of 20mg/750mL basic fuschin dye by *Saccharomyces cerevisiae***

Figure 1, 2 and 3 showed the effects of incubation period on the decolourizations of basic fuschin dye with different concentrations at 20mg/250mL, 20mg/500mL and 20mg/750mL dilutions of the dye respectively by *Saccharomyces cerevisiae*. The level of decolourization increased as the incubation period increased from 3 to 12 days at three days interval. The maximum decolourizaton occurred at 20mg/750mL and the minimum occurred at 20mg/500mL dye concentrations. Daivasigamani and Das[13] reported a remarkable decrease in intensity of the treated basic violet-3 dye with increasing incubation period using the predicted equation in their work on biodecolourization of basic violet-3 by *Candida krusei* isolated from textile wastewater.

## CONCLUSION

The isolated yeast, *Saccharomyces cerevisiae* was capable of degrading basic fuschin dye with the dye as sole carbon source. Hence, *Saccharomyces cerevisiae* can serve as promising microorganism for the treatment of effluents containing basic fuschin dye. However, the decolourization of the basic fuschin dye increase with increase in incubation period.

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