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BIODECOLORIZATION OF BASIC FUCHSIN DYE BY *Saccharomyces cerevisiae* ISOLATED FROM SALT WATER AND PALM WINE

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author AHS developed the concept, managed the literature searches and carried out experiments. Author AOP designed the experiments. Author DD carried out the isolation and identification author AAS carried out microscopy and designed the charts. Author AJO performed decolourization assays. All authors read and approved the final manuscript.

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ABSTRACT

In this study, *Saccharomyces cerevisiae* isolated from palm wine and salt water was used to degrade 20 mg basic fuchsin dye for a period of 12 days under aerobic condition in 250 mL, 500 mL and 750 mL mineral salt media. The degree of decolorization of basic fuchsin was determined using UV-visible spectrophotometer with absorbance of 620 nm. At the end of twelve days, 60.39%, 41.29% and 24.47% (for *Saccharomyces cerevisiae* isolated from salt water) basic fuchsin decolorization by *Saccharomyces cerevisiae* were recorded and 72.61%, 48.88% and 33.92% (for *Saccharomyces cerevisiae* isolated from palm wine) basic fuchsin decolorization by the same organism were recorded in 250 mL, 500 mL and 750 mL concentrations at pH 6.5, respectively. The results suggest the potential of *Saccharomyces cerevisiae* for the treatment of waste water containing basic fuchsin.

Keywords: Basic fuchsin; dye; spectrophotometer; decolorization; *Saccharomyces cerevisiae*.

1. INTRODUCTION

Basic fuchsin is a triphenylmethane dye made up of some chemically related dyes such as Basic red 9 (Magenta O), Magenta I (Rosaniline), Magenta II, and Magenta III (New fuchsin). The dye was among the first to be produced beginning in the 1850s [1]. Magenta is used in hair dye, as a colorant in artist paints, as cosmetics products not intended to come in contact with mucous membranes, to stain 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. Rehn [1] was the first to report the appearance of bladder tumors in three of 45 workers involved in the

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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 [1]. The microbial decolorization of dyes has been of considerable interest due to their inexpensive and ecofriendly nature as well as producing a less amount of sludge [5]. Presently, an extensive research has been 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 [6]. 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. Biodegradation is defined as the biologically mediated breakdown of chemical compounds into various byproducts through the action of various enzymes. Many researchers have reported the bio decolorization of various dyes using organisms obtained from different extracts. For example Yatime et al. [7] reported the decolorization of crystal violet by *Actinomycetes* such as *Nocardia coralline* and found out that the dyes were completely decolorized in 24 hours. They also reported the decolorization of four triphenylmethane dyes such as basic fuchsin, methyl violet, crystal violet, and Victoria blue by bacteria, *Pseudomonas pseudomallie* and found out that methyl violet and crystal violet were appreciably decolorized while basic fuchsin and Victoria blue were not decolorized under experimental conditions. Kwaniewska [8] showed that oxidative yeast such as *Rhodotorula sp.* and *Rhodotoru larubra* were capable of degrading crystal violet in liquid broth. The biodecolorization of basic fuchsin by yeast extracted from palm wine and saltwater was not undertaken. The structural, molecular and relative atomic mass of basic fuschin dye is shown below:

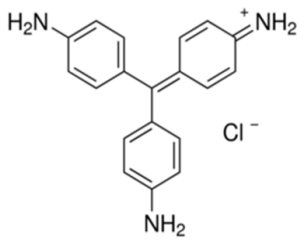

Fig. 1. Chemical structure of basic fuchsin

Wastewaters containing residues of basic fuchsin dye from industries and laboratories wash down into flowing streams and rivers and pose serious health hazard to the receiving communities. There is therefore the need to degrade the dye before their eventual passage into flowing stream or rivers [8]. Chemical processes are extensively used for the degradation of dyes by different dye manufacturing and dyeing industries [9]. However, these processes are not safe to use as recalcitrant. Biological processes are encouraged because they are safer and environmentally friendly [1]. The aim of this research study was to carry out biodecolorization of basic fuchsin dye by yeast isolated from Palm wine and Saltwater.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preservation

The basic fuschin dye used in this study was obtained from the department of Biological Sciences Federal University of Technology Minna, Nigeria. A commercially prepared palm-wine was bought from Tunga and the salt-water was obtained from Oniru beach in Lagos State, Nigeria and was kept in a sterile bottle and stored at 4ºC. Other chemicals used in the analysis were of analytical grade.

2.2 Sterilization Techniques

All glasswares were washed with detergent, rinsed thoroughly with distilled water and oven dried at 160ºC for one hour. All media and solutions prepared were sterilized by autoclaving at 121ºC for 15minutes.

2.3 Isolation and Identification of Yeast Isolates

Yeast was isolated from Palm-wine and Salt-water using pour plate technique. Serially diluted sample of Palm-wine and Salt-water was plated on sabouraud dextrose agar (SDA) and incubated at ambient laboratory temperature (28ºC 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 method of Abdullahi et al. [10] based on their morphological characteristics. The isolated yeast colonies were maintained on 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 and *Saccharomyces cerevisiae* was comfirmed (Table 1) [11].

Table 1. Biochemical and microscopic comfirmation test for *S. cerevisiae*

Colonial Morphologyon SDA	Microscopic	Sugar fermentation					
		Glucose	Lactose	Raffinose	Galactose	Fructose	Sucrose
Smooth, Moist, Cream in color	No pseudomycelia, Budded, large globose, no hypae	+	--	+	+	+	+

2.4 Sugar Fermentation Test

The isolated yeast was tested for the ability of ferment sugars such as glucose and sucrose using the solution such as 1% of peptone, 0.1% of NaCl and Durham's tube. The Durham's tube was inverted in the test tube containing broth media, glucose or sucrose, and the solutions mentioned above, which was incubated at ambient laboratory temperature.

2.5 Nitrate Reduction Test

Nitrate broth composing of KNO_3 and peptone was prepared, dissolved in distilled water in a conical flask, autoclaved and dispersed in Sterile Petri-dishes, inoculated with the test organisms. 0.1mL of Sulphanilic acid was added and incubated. No colour changes were observed indicating that the yeasts were not able to utilize nitrate. The suspension cultures of yeast were used to investigate their abilities to degrade basic fuschin dye. They were prepared in nutrient broth and incubated at ambient laboratory temperature for 48 hours [12].

2.6 Decolorization of Basic Fuschin Dye by Yeast

The decolorization media (mineral salt) was prepared using 1Liter of distilled water according to Deivasgamani and Das [13], with a little modification. The mineral salt medium was composed of yeast extract-2.0gL^{-1}, NaCl-0.5gL^{-1}, NH_4Cl-1.0gL^{-1}, $MgSO_4$- 3gL^{-1}, $(NH_4)_2SO_4$ 0.3gL^{-1}, KH_2PO_4- 0.5gL^{-1}, $NaHCO_3$- 1.0gL^{-1}, $CaCl_2.6H_2O$- 0.2gL^{-1}, $NaB_2O_7.10H_2O$-0.2gL^{-1}, $MnCl_2.4H_2O$-0.1gL^{-1}, $ZnSO_4.7H_2O$-0.1gL^{-1}, and $CuSO_4.5H_2O$. 20 mg of basic fuschin dye was added to 250 mL, 500 mL and 750 mL 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 121ºC for 15 minutes.

After 24 hours, 2ml of the cell cultured in nutrient broth media were inoculated into eight (8) conical flask containing 20mL of the growth media and basic fuschin dye in 50 mL 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 fuchsin dye was taken at 630 nm using UV-spectrophotometer (M^R 752 UV –spectrophotometer, Model YM1208PTS1) after centrifugation at 1200 rpm for 15 minutes. The experiment lasted for 12 days and absorbance was taken at three days interval alongside the control, which was necessary in order to monitor the decolorization of the dye by the yeast isolate.

2.7 Decolorization Assay

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

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

Where Ao and At represents initial and final dye concentrations in mgL^{-1}, respectively.

3. RESULTS AND DISCUSSION

The isolated strains were subjected to biochemical methods for species identification. The biochemical methods were based on the utilization of carbon and nitrogen sources. The yeast isolates isolated from palmwine and salt water samples were identified by microscopic and biochemical studies as belonging to *Saccharomyces* species. The isolates showed good decolorization ability for basic fuchsin dye. Fig. 2 shows the percentage degradation of basic fuschin dye by *Saccharomyces cerevisiae* isolated from salt water at different incubation periods in 20 mg/ 200 mL. The dye was degraded effectively on day 12 with 60.39% degradation. On day 3, 48.09% degradation was recorded while between day 6 and 9, it was found to be 55.93% and 56.02%, respectively.

Fig. 3 shows the percentage degradation of basic fuschin dye by *Saccharomyces cerevisiae* isolated from palm wine in 20 mg/250 mL at different incubation periods. The dye was degraded effectively on the 12th day with 72.617%. On the 3rd day 58.03% degradation was recorded while between the 6th and 9th day it was found to be 59.48% and 66.91%, respectively.

Biodegradation of basic fuchsin dye was studied in batch culture using *Saccharomyces cerevisiae* isolated from palm wine and salt water. In order to test the activity of the isolated organisms on the dye, the experiments were carried out in flasks. *Saccharomyces cerevisiae* isolated from palm wine was found to be the most effective decolorizer of basic fuchsin (Fig. 3).

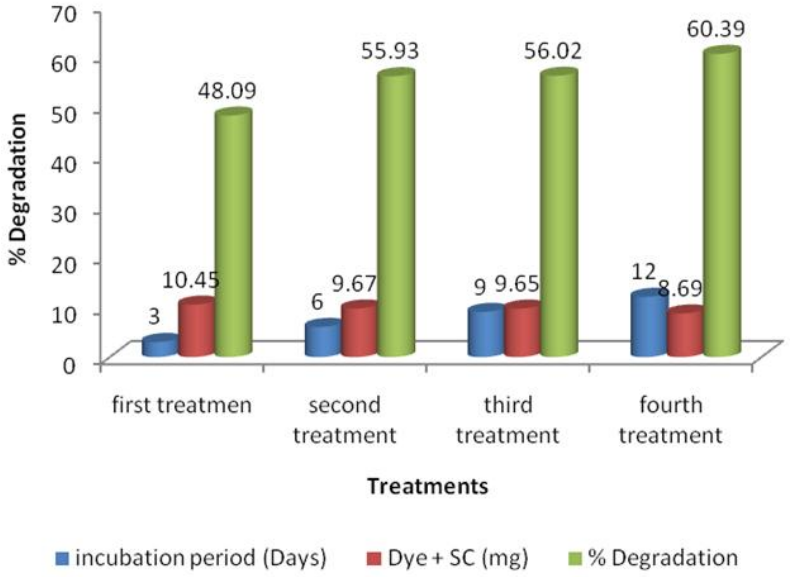

Fig. 2. Degradation of basic fuschin dye by yeast (*Saccharomyces cerevisiae*) isolated from salt water in 20 mg/250 mL

Key: SC=Saccharomyces cerevisiae

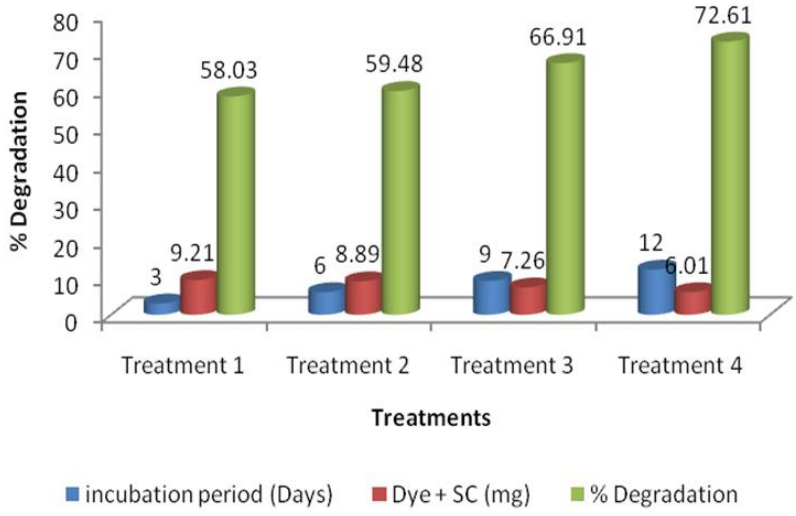

Fig. 3. Degradation of basic fuschin dye by yeast (*Saccharomyces cerevisiae*) isolated from palm wine in 20 mg/250 mL

Key: SC=Saccharomyces cerevisiae

The highest percentage degradation (72.61%) was obtained from *Saccharomyces cerevisiae* isolated from palm wine (Fig. 3). This could have been due to the activeness of the isolate because palm wine is very rich in vitamins, protein, thiamine and riboflavin, which are nutrients that are required by *Sacchromyces cerevisiae* for growth and metabolism.

The percentage degradation of basic fuschin dye in 20 mg/500 mL at different incubation periods is shown in Fig. 4. The dye was degraded effectively on the 12th day with 41.29% on the 3rd day 8.71% degradation was recorded while between the 6th and 9th day it was found to be 10.64% and 37.13%, respectively.

The percentage degradation of basic fuschin dye in 20 mg/500 mL at different incubation periods is shown in Fig. 5. The dye was degraded effectively in day 12 with 48.88%. In day3, 25.45% degradation was recorded while between the 6th and 9th day it was found to be 26.12% and 48.21, respectively.

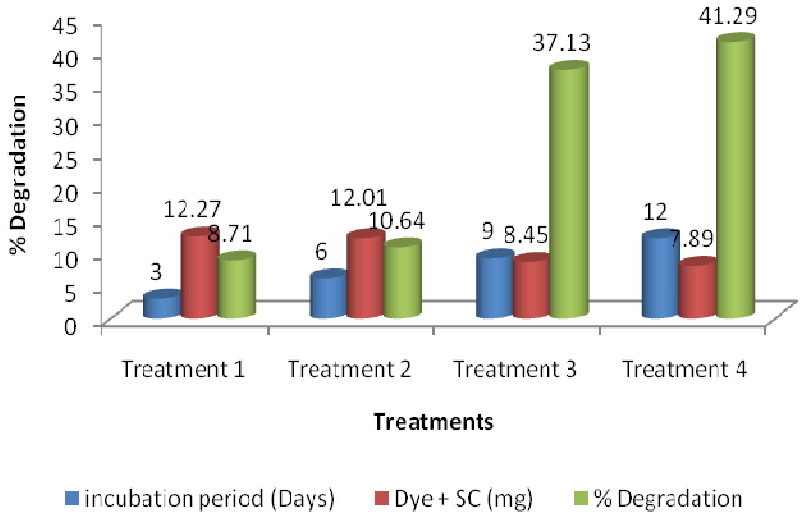

Fig. 4. Degradation of basic fuschin dye by yeast (*Saccharomyces cerevisiae*) isolated from salt water in 20 mg/500 mL

Key: SC = Saccharomyces cerevisiae

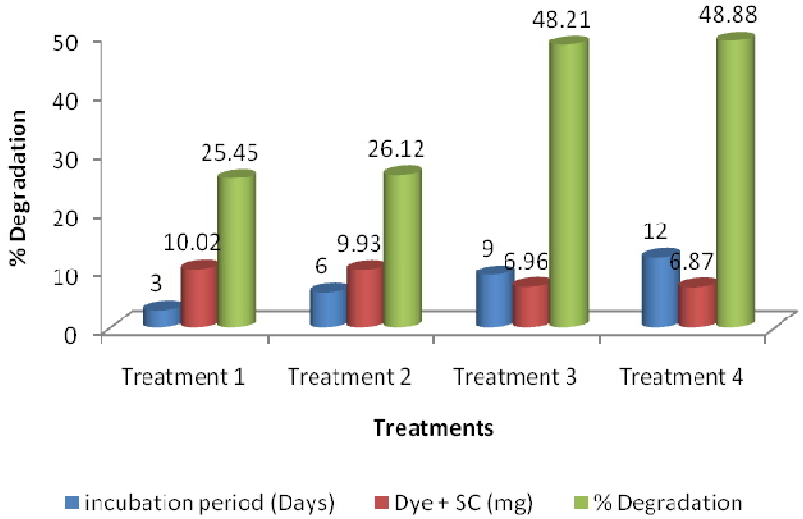

Fig. 5. Degradation of basic fuschin dye by yeast (*Saccharomyces cerevisiae*) isolated from palm wine in 20 mg/500 mL

Key: SC=Saccharomyces cerevisiae

Fig. 6 shows the percentage degradation of Basic fuschin dye at different incubation periods in 20 mg/750 mL. The dye was degraded effectively on the 12th day with 24.47% degradation. On the 3rd day 10.17% degradation was recorded while between the 6th and 9th day it was found to be 12.65% and 13.12%, respectively.

Fig. 7 shows the percentage degradation of basic fuschin dye in 20 ml/ 750 mL at different incubation periods. The dye was degraded effectively on the 12th day with 33.92% on the 3rd day 3.19% degradation was recorded while between the 6th and 9th day it was found to be 24.47% and 26.71%, respectively.

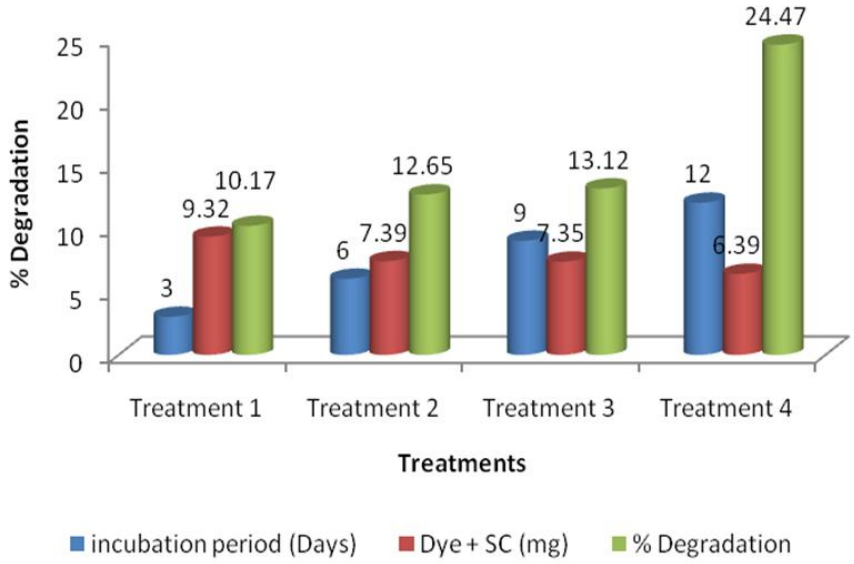

Fig. 6. Degradation of basic fuchsin dye by yeast (*Saccharomyces cerevisiae*) isolated from Salt water in 20 mg/750 mL

Key; SC=Saccharomyces cerevisiae

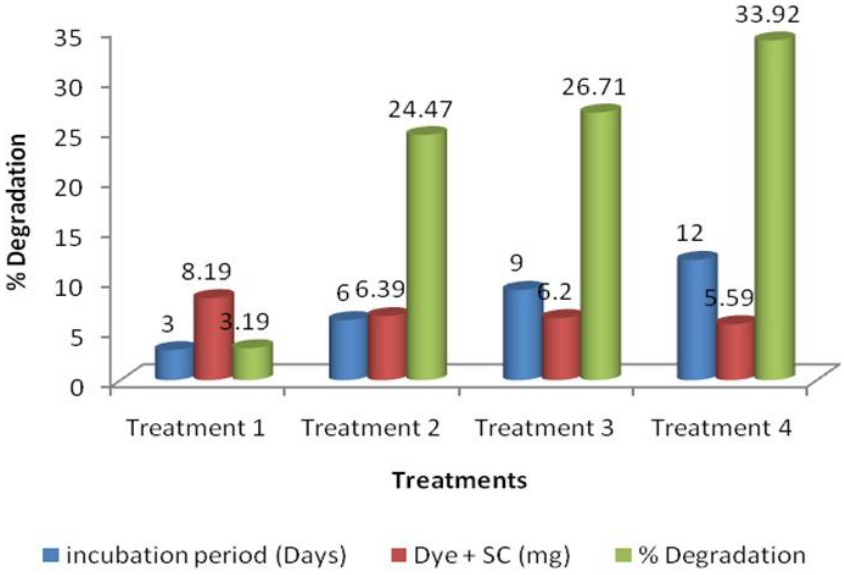

Fig. 7. Degradation of basic fuschin dye by yeast (*Saccharomyces cerevisiae*) isolated from palm wine in 20 mg/750 mL

Key; SC=Saccharomyces cerevisiae

The yeast (*Saccharomyces cerevisiae*) isolated from salt water decolorized basic fuschin dye in the range of 48.09 to 60.39% (250 mL), 8.71 to 41.29% (500 mL) and 10.7 to 24.47% (750 mL) between 3 to 12 days incubation period under (Figs. 1, 3 & 5) while that isolated from palm wine decolorized basic fuschin dye in the range of 58.03 to 72.61% (250 mL), 25.45 to 48.88% (500 mL) and 3.19 to 33.92% (750 mL) between 3 to 12 days incubation period under aerobic condition (Figs. 3, 5 and 7). The higher percentage of decolorization of *Saccharomyces cerevisiae* isolated from palm wine could be that the palmwine serve as a very good nutrient source for *Saccharomyces cerevisiae* which could be seen by the larger number of growth of the organism. Similar results were reported on biodegradation of azo dyes by yeast *(Issatchenkia occidentails*), were maximum decolorization was observed under aerobic conditions [15]. They further reported that under anoxic condition decolorization was lesser due to the absence of metabolic activities. Also, Daivasigamani and Das [13], in their work on biodecolorization of basic violet-3 by *Candida krusei* isolated from textile waste water, reported maximum decolorizations of 74% and 100% in the media supplemented with sucrose and sugarcane bagasse extract within 24 hours, respectively. Also, similar observations were also made by Vitor and Corso [16], who recorded 73.2% maximum degradation of textile dye by *Candida albicans.*

Adsorption of dyes to the microbial cell surface is the primary mechanism of decolorization [17]. The dye degradation/decolorization of this study has previously been reported by various researchers [18] [19]. Decolorization of various dyes is related to the various processes of extracellular oxidases, such as manganese peroxideases [20].

The decolorization in 20 mg/250 mL prepared culture recorded the highest percentage degradation and increased with increase in incubation period from 3 to 12 days using the yeast (*Saccharomyces cerevisiae*) isolated from salt water and palm wine. The concentration of 20 mg/500 mL showed decolorization increasing as the incubation period increased. For 20 mg/750 mL, the decolorization also increased with increase in incubation period up to 12 days. 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 biodecolorization of basic violet-3 by *Candida krusei* isolated from textile waste water.

The results show 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, biodecolorization of textile dye effluent by *Aspergillus species, Fusarium oxysporum*, *Penicillium chrysogenum*, *Mucor species* and *Trichoderma viridei*.

4. CONCLUSION

The isolated yeast, *Saccharomyces cerevisiae* from salt water and palm wine were 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. The dye decolorizing/degrading activity of the yeast can be further analyzed deeper in order to develop an eco-friendly remediation technique for the remediation of textile dyes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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