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Microbial Decolourization of an Anthraquinone Dye C.I. Reactive Blue 19 Using *Bacillus cereus*

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Authors' contributions

This work was carried out in collaboration between all authors. AG and FJG designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. BJI managed the analyses of the study. AG managed the literature searches.

All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: This work aimed at using *B. cereus* strain to decolourize a textile dye and also to study the influence of various environmental parameters on the decolourization processes. **Study Design:** Decolourization efficiency of *B. cereus*.

Place and Duration of Study: Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria; Department of Medical Microbiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria and Department of Textile Science and Technology, Ahmadu Bello University, Zaria, Nigeria, between March 2011 and September 2011.

Methodology: The *B. cereus* strain used was isolated from contaminated food by using a selective media (Mannitol egg yolk polymyxin agar) and then culturing and storing on nutrient agar slants at -20° C after biochemical tests were done to identify the isolate. All the microbial batch experiments were carried out at ambient conditions in 250ml Erlenmeyer flasks. Nutrient broth was autoclaved at 121°C at 15psi for 15min and nutrient agar plates were also used in the isolation of the *B. cereus* strain. Effects of various parameters, including initial dye concentration (0, 0.5, 1.0 and 2.0g/l), glucose concentration (50, 100, 200 and 500mg/l), pH (4.0, 7.0 and 10.0) and temperature (20, 27 and 40°C), on dye decolourization were investigated. Decolourization extent was determined by measuring the absorbance of the culture supernatant at 591nm using a Unicam UV9100-visible spectrophotometer. Decolourization extent was calculated using a

standard equation as specified by Giwa et al., 2011.

Results: The bacteria culture exhibited 95% decolourization ability within 72 hours. The optimum dye decolourizing activity of the culture was observed at pH 7.0 and incubation temperature of 27°C. Maximum dye decolourizing efficiency was observed at 200 mg/l concentration of RB19. The dye solution showed high peak at the wavelength of 591nm.

Conclusion: The results thus obtained have characterized and identified the dye degrading ability of the *B. cereus*. The presence of a co-substrate (glucose) is an essential condition for attaining maximum decolourization efficiency. Reactive blue 19 was completely and rapidly decolourized by *B. cereus* after 3days of incubation with different effects on the dye as seen in the result.

Keywords: Microbial oxidation; anthraquinone dye; C.I. reactive blue 19; decolourization; B. cereus.

1. INTRODUCTION

Synthetic dyes are widely used in the textile, cosmetic, printing, drug and food processing industries (Padamavathy et al., 2003). The generation of coloured wastewaters is a problematic reality for a variety of industrial sectors. Among these are effluents released from textile and printing processes, dry cleaning and tanneries, the food industries, manufacture of paints and varnishes, manufacture of plastics and a variety of chemical processes.

Insufficient treatment of wastes of the dyestuff industries leads to dye contamination of the environment such as soil and natural water bodies (Pearce et al., 2003). Commercial dyes have variety of colours and a high stability to light, temperature and microbial attack. Not only is the colour aesthetically unacceptable, it also affects aquatic ecosystem by decreasing the light penetration and solubility of gases (Saranaik and Kanekar, 1995). Furthermore, some synthetic dyes such as azo dyes are carcinogenic or mutagenic (Spadaro et al., 1992). Physico-chemical treatment processes have disadvantages in that the contaminant is not destroyed, it is simply concentrated and subsequently deposited in landfills or incinerated, while biological treatment methods are cheap and offer the best alternative with proper analysis and environmental control (Banat et al., 1996).

One promising strategy is the use of microbes that possess the ability to decolourize synthetic dyes including white rot fungal and bacterial strains (Liu et al., 2004; Hadibarata et al., 2012a, 2012b). Microbial decolourization and degradation is an environmentally friendly and cost-competitive alternative to physico-chemical decomposition processes for the treatment of industrial effluents (Verma and Madamwar, 2003).

Many bacterial, fungal and algal species have the ability to absorb and/or degrade textile dyes. Bacteria decolourization of azo dyes is either aerobic or anaerobic (Forgacs et al., 2004; Pandey et al., 2007). Nature is full of bacterial diversity. Azo dye decolourizing bacteria can be isolated from soil, water, human and animal excreta and even from contaminated food materials. However, other potential ecological niches for isolating such bacteria are coloured effluents arising from dye manufacturing and textile industries.

This work aimed at using *B. cereus* strain to decolourize a textile dye and also to study the influence of various environmental parameters on the decolourization processes.

2. MATERIALS AND METHODS

2.1 Microbial Oxidation System of Decolourizing Bacteria

The *B. cereus* strain used was isolated from contaminated food by using a selective media (Mannitol egg yolk polymyxin agar) and then culturing and storing on nutrient agar slants at – 20°C after biochemical tests were done to identify the isolate in the department of Microbiology, Ahmadu Bello University, Zaria, Nigeria. All the microbial batch experiments were carried out at ambient conditions in 250ml Erlenmeyer flasks. Nutrient broth (100ml of distilled water containing peptone 0.5g, yeast extract 0.3g, beef extract 0.2g, sodium chloride 0.5g) was autoclaved at 121°C at 15psi for 15min and nutrient agar plates were also used in the isolation of the *B. cereus* strain.

Colonies of *B. cereus* growing on nutrient agar slants were inoculated into sterile 10ml nutrient broth in universal bottles to make the bacterial suspension. This was cultured on sterile nutrient agar plates and incubated at 37 for 24 hours to obtain discrete colonies. After the period of incubation, characteristically large creamy colonies growing on nutrient agar plates were identified by morphological, cultural and biochemical tests following the current method recommended in the US Food and Drugs Administration Bacteriological Analytical Manual (BAM 2) (Rhodehamel et al., 2001)

2.2 Effects of Different Parameters on Dye Decolourization

The bacteria was cultured in LB medium at 150 rpm, 37°C for 12hours before the bacterial cells were collected by centrifugation (5000 rpm for 5 min) and re-suspended in modified M9 synthetic medium. The bacterial suspension was inoculated in 100ml Erlenmeyer flasks containing 60ml modified M9 medium to study the effect of the conditions at 150rpm and 37°C. Effects of various parameters, including initial dye concentration (0, 0.5, 1 and 2g/l), glucose concentration (50, 100, 200 and 500mg/l), pH (4.0, 7.0 and 10.0) and temperature (20, 27 and 40°C), on dye decolourization were investigated. Experiments were performed in a 15ml glass tubes containing 10ml of the medium. The bacterial cell suspension was inoculated into the tubes and the optical density (600 nm) was 0.3. Each experiment was carried out in triplicate, and the average was recorded.

2.3 Bacteria Decolorization of Reactive Blue 19

The bacterial cell suspension was autoclaved at 121°C at 15psi for half an hour and then added into the medium and the decolourization extent tested. Bacterial cells without treatment were added into the medium as a control. Both the experiments of decolourization by treated and untreated bacterial cells were performed under anaerobic conditions. After incubation for 48hours, samples from the control culture were centrifuged at 5000rpm for 5min and the supernatant was scanned from 300–700nm using a Unicam UV9100-visible spectrophotometer to detect any transformation of compounds in the medium.

2.4 Measurement of Decolourization Extent

Samples (0.4ml) were collected every 24 hours and centrifuged at 5000rpm for 5 min. Decolourization extent was determined by measuring the absorbance of the culture supernatant at 591nm using a Unicam UV9100-visible spectrophotometer. Decolourization extent was calculated using the following equation 1 (Giwa et al., 2011):

Decolorization extent (%) =
$$\frac{C_o - C_t}{C_o} \times 100$$
 Equation 1

Where C_o refers to the initial absorbance, C_t refers to the absorbance after incubation; and t refers to the incubation time.

2.5 Statistical Analysis

The knowledge that any individual measurement that is made in the laboratory may lack perfect precision has led us to choose to take multiple measurements of decolourization efficiency. Though no one of these measurements are likely to be more precise than any other, this group of values, it is hoped, will cluster about the true value we are trying to measure. The distribution of data values is represented by showing a single data point, representing the mean value of the data and error *bars* to represent the overall distribution of the data. These error bars are graphical representation of the variability of our data and are used on respective graphs to indicate the error, or uncertainty in our reported decolourization efficiency measurement. Error bars give a general idea of how accurate a measurement is, or conversely, how far from the reported value the true (error free) value might be. However, here in our report, error bars represent 95% confidence interval. It is worthy to mention that instead of creating a graph using all of the raw data, now only the mean value is plotted with error bars for percentage decolourization.

3. RESULTS AND DISCUSSION

The properties of the dye used are shown in Table 1.

3.1 Effect of Dye Concentration of RB19

Decolourization activity of the bacterial culture of *B. cereus* was studied using Reactive blue 19 at different concentrations varying from 0.0g/l to 2.0g/l (Fig. 1). Rate of decolourization increased with increase in initial dye concentration up to 1.0g/l (97.89% decolourization). Further increase in dye concentration resulted in reduction in decolourization rates. A survey of the literature suggests that increasing the dye concentration gradually decreases the decolourization rate, probably due to the toxic effect of dyes with regard to the individual bacteria and/or inadequate biomass concentration (or improper cell to dye ratio), as well as blockage of active sites of azoreductase by dye molecules with different structures (Jadhav et al., 2008; Saratale et al., 2009a).

Similar results were observed in the bacterial decolourization of various reactive azo dyes (Saratale et al., 2009b). It was also observed that reactive group azo dyes with sulfonic acid (SO_3H) groups on their aromatic rings greatly inhibited the growth of microorganisms at higher dye concentrations (Kalyani et al., 2008).

Table 1. Properties of RB19

Property	Dye
C. I. Name	Reactive Blue 19
Commercial Name	Remazol Brilliant Blue R
Functional group	Anthraquinone
CAS Number	2580-78-1
C.I. Number	61200
Mol. Mass (g/mol)	626.54
Mol. Formula	$C_{22}H_{16}N_2Na_2O_{11}S_3$
Chemical Formula	O NH2 O SONA O HN SONA O SONA
Lambda max (nm)	591
Purity (%)	99
Solubility	30 g/L in water @ 80°C
Manufacturer	SIGMA
pH of stock solution	6.524 @ 31.3°C

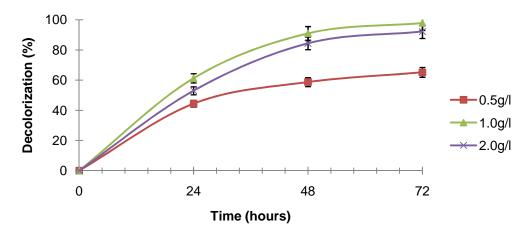


Fig. 1. Dye Concentration Curve for RB19 and each data point is the average of triplicates and the error bars represent 95% confidence interval

3.2 Effect of Glucose Concentration on Decolourization of RB19

The decolourization efficiencies were almost the same as the concentration of glucose was increased from 50 to 200mg/l (Fig. 2). The colour removal efficiencies reached a peak resulting in a decolourization efficiency of 99% after 3 days of incubation period. However, 50% decolourization removal was obtained for concentration of 500mg/l after 3days of incubation. In this study, our strain required sugar especially, glucose for decolourization. This result seems to suggest that the decolourization activity of this strain might be a sugar

oxidase as the same with Coriol us sp. No.20 (Watanabe et al., 1982) and C. versi colour Ps4a (Ohmomo et al., 1988).

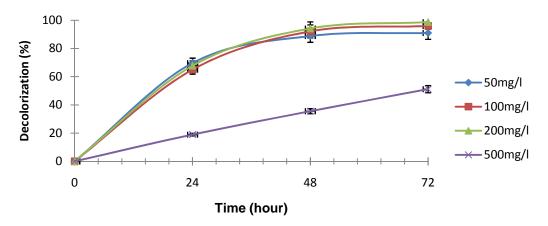


Fig. 2. Glucose Concentration Curve for RB19 and each data point is the average of triplicates and the error bars represent 95% confidence interval

3.3 Effect of pH on Decolourization of RB19

Bacterial culture generally exhibits maximum decolourization rate at pH values near 7. Decolourization of C.I. Reactive Blue 19 at various pH values by *B. cereus* was studied. Fig. 3 shows that an increase in pH from 4.0 to 7.0 gives 19.98% and 95.99% decolourization respectively, while decolourization rate decreased as pH was increased from 7.0 to 10.0, a concomitant reduction in decolourization from 95.99% to 76.67% was observed. The rate of decolourization for *B. cereus* was optimum in this narrow pH range.

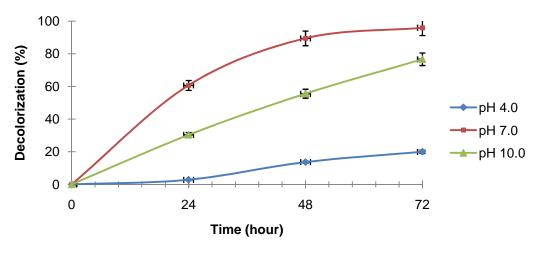


Fig. 3. pH Curve of decolourization of RB19 and each data point is the average of triplicates and the error bars represent 95% confidence interval

These results show that the pH of the medium is also an important factor with regards to decolourization. The rate of colour removal is higher at the optimum pH, and tends to

decrease rapidly at strongly acid or strongly alkaline pH. It is thought that the effect of pH may be related to the transport of dye molecules across the cell membrane, which is considered as the rate limiting step for decolourization (Chang et al., 2001a; Kodam et al., 2005). This pH tolerance of decolourizing bacteria is quite important, as it makes them suitable for practical bio-treatment of dyeing mill effluents (Aksu and Donmez, 2003; Wang et al., 2009).

3.4 Effect of Temperature on Decolourization of RB19

In microorganisms the environmental temperature directly establishes organismal temperature, as the microbial cell responds to temperature changes by adaptation via biochemical or enzymatic mechanisms. Consequently, temperature is a factor of paramount importance for all processes associated with microbial vitality, including the remediation of water and soil. The dye decolourization activity of our culture was found to increase with increase in incubation temperature from 20 to 27°C with maximum activity attained at 27°C (92.34% de colourization) Fig. 4. Further increase in temperature resulted in marginal reduction in decolourization activity of culture *B. cereus*. it has also been reported that in microbial physiology temperature changes lead to a sudden alteration of the activation energy (Yu et al., 2001). Moreover, the effects of temperature on the growth rate, biomass yield and reaction mechanism have also been reported (Blaga et al., 2008).

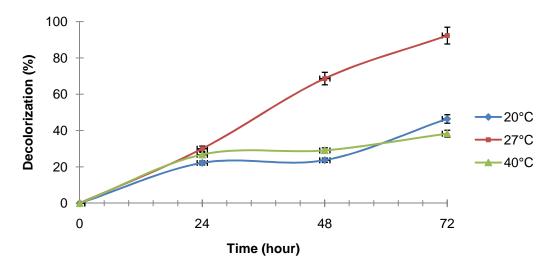


Fig. 4. The Effect of Temperature on Decolourization of RB19, and each data point is the average of triplicates and the error bars represent 95% confidence interval

4. CONCLUSION

The present study confirms the ability of *B. cereus* to decolourize the Reactive blue 19 with a decolourization efficiency of 95%. The presence of a co-substrate (glucose) is an essential condition for attaining maximum decolourization efficiency. The results thus obtained have characterized and identified the dye degrading ability of the *Bacillus*. The *Bacillus* strain has the ability to tolerate, decolourize and degrade azo dyes even at high concentration. Reactive blue 19 was completely and rapidly decolourized by *Bacillus* after 3days of incubation with different effects on the dye as seen in the result.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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