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Full Length Research Paper

Aerobic decolourization of two reactive azo dyes under varying carbon and nitrogen source by *Bacillus cereus*

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***Bacillus cereus* isolated from dye industrial waste, that is, effluent and soil samples was screened for its ability to decolourize two reactive azo dye – cibacron black PSG and cibacron red P4B under aerobic conditions at pH 7 and incubated at 35°C over a five day period. Different carbon and nitrogen sources were used for the decolourization study. *B. cereus* was able to decolourize cibacron red P4B by (81%) using the combination of ammonium nitrate and sucrose, while it decolourizes cibacron black PSG by (75%) using yeast extract and lactose.**

Key words: Bioremediation, decolourization, textile dye, *Bacillus cereus.*

INTRODUCTION

Dyes are organic chemical compounds, which impart colour to other materials by saturating them in aqueous solution. Synthetic dyes have a wide application in the food, pharmaceutical, textile, leather, cosmetics and paper industries due to their ease of production, fastness and variety in colour compared to natural dyes. More than 100000 commercially available dyes are known and close to one million tons of these dyes are produced annually worldwide (Adedayo et al., 2004).

Dyes are designed to remain stable and long-lasting colourants; they are usually not easily biodegraded. Dye colours are visible in water at concentration as low as 1 mg/L, whereas textile processing waste water, normally contain more than 10-200 mg/L dye concentration, resulting in aesthetic problems (O'Neil et al., 1999).

The toxicity of dye industrial waste effluents to life, including human being has been described (David et al., 1988; Kanekar et al., 1993). It is therefore necessary to treat the dye containing waste effluent before discharging into the receiving water.

Several methods are used in the treatment of textile effluents to achieve decolourization. These include physio-chemical methods such as filtration, specific coagulation, use of activated carbon and chemical flocculation. Some of these methods are effective but quite expensive and have many disadvantages and limitations (Do et al., 2002; Maier et al., 2004). It is therefore, important to develop efficient and cost-effective methods for the decolourization and degradation of dyes in industrial effluents and contaminated soil (Bhatt et al., 2000).

Bioremediation offers a cheaper and environmentally friendlier alternative for colour removal in textile effluents. The ubiquitous nature of bacteria makes them invaluable tools in effluent biotreatment. Several reports have been published on bacterial azo dye reduction under different conditions (Hu, 2003; McMullan et al., 2001; Stolz, 2001).

Azo dyes generally resist aerobic microbial degradation, only organisms with specialized azo dye reducing enzymes were found to degrade azo dyes under fully aerobic conditions (Ganesh et al., 1994). Aerobic metabolism of dyes by *Pseudomonas mendocrina* M2M B-404 and *Sphingomonas xenophaga* BN6 is studied by Sarnaik and Kanekar (1999) and Stolz (1999), respectively. Studies by Buitron et al. (2004) with Acid red 151 azo dyes under aerobic conditions using a microbial consortium led to 99% colour removal.

In many Nigerian cities, the textile factories daily discharge millions of litres of untreated effluents in the form of wastewater into public drains that eventually empty into rivers (Olayinka and Alo, 2004).

Dyeing of textile fabrics is a popular cottage industry in Abeokuta, Nigeria, where the waste effluents are discharged untreated into the environment. The removal of

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polluting dyes in Abeokuta city poses a major problem due to the traditional small holding nature of the business. Economically, this does not encourage the siteing of a municipal waste treatment plant. Therefore, the use of microbial communities for on-site treatment of dye containing waste waters from textile and dye-stuff industries could be an economical alternative. This research is therefore aimed at investigating the potential of locally isolated bacterial spp in the decolourization of textile dyes and also the effects of varying nutrient sources on the aerobic decolourization of two reactive azo dyes. This is done in-order to determine the optimal decolourization parameters.

MATERIALS AND METHODS

Dyes

Five different textile dyes; reactive torquoise blue, disperse yellow, reactive orange H3R, cibacron red P4B and cibacron black PSG were used for both the screening and final experiment. All dyes were procured from United Nigeria Textile Mill PLC, Ibese Road, Ikorodu, Lagos, Nigeria. Dye stock solutions were prepared by dissolving 5.0 mg of each dye in 0.09% (w/v) NaOH.

Determination of the dye maximum absorbance

The maximum absorbance of each dye was determined spectrophotometrically using (Jenway, 640s UV/VIS spectrophotometer); Cibacron black PSG (555 nm), Cibacron red P4B (543 nm), Disperse yellow (520 nm), Reactive Torqouise blue (520 nm), Reactive orange H3R (600 nm).

Collection of samples

Textile effluents and soil samples from effluents sites were collected at random in duplicates from two different sites in Itoku market, Abeokuta, Ogun state and University of Agriculture, Abeokuta textile mill, in sterile plastic bottles.

Isolation of microorganisms

Microorganisms were isolated from the textile effluents and soil samples by preparing aliquot (10 ml) dilutions of textile effluents and soil samples. Nine milliliters of sterile water was placed in McCartney bottles and labeled 10^{-1} to 10^{-6}, after which 1 ml sterile pipette was used to transfer 1 ml of effluent sample into each of them. One gram (1 g) of soil was also transferred into already prepared aliquot samples like that of the above. 1 ml was then taken from both soil and effluent aliquots and plated on dye fermentation agar medium containing cibacron black PSG and incubated aerobically at 35°C for 72 h. Cultures capable of growth on this medium were isolated and purified by sub-culturing on dye fermentation agar medium (Hu, 1994).

The purified isolates were characterized by standard microbiological methods and identified according to Buchanan and Gibbons (1986).

Preparation of dye decolourization medium

Dye stock solution of each dye were filter-sterilized separately on membrane filter, pore size of 0.2 μm (Millipore, USA). 5 mg of dye stock solution was added to 1 litre of the medium containing the following ingredients: $MgSO_4\ 2H_2O$ (0.1%), KH_2PO_4 (0.05%), NH_4No_3 (0.1%), $CaCl_2$ (0.1%), $FeSO_4$ (0.05%) and glucose (0.05%) as described by Merchant et al. (1994) to make dye fermentation medium.

Culture conditions for decolourization

Dye-fermentation media (50 ml), containing each of 5 different dyes in 100 ml Erlenmeyer flask was prepared in duplicate. About 1 ml from 24 h old broth culture of 3 different bacteria were inoculated into the flask, that is, *Bacillus cereus, Micrococcus acidophilus* and *Streptococcus faecalis*. Uninoculated flask served as control. The flasks were incubated aerobically at 35°C for 5 days on an orbital shaker at 200 rpm. While anaerobic flasks were sealed with sterile subseals and incubated in anaerobic jars for 5 days. Samples were withdrawn at 24 h intervals for centrifugation (4000 rpm for 20 min) and analyzed for visible spectra of each dye spectrophotometrically.

Variation of nutrient sources

The dyes and organisms that show better decolourization ability was then subjected to variation of nitrogen and carbon sources to see its effect on the decolourization ability under aerobic conditions. Ammonium nitrate (NH_4NO_3), peptone and yeast extract serve as the different nitrogen sources used, while glucose, lactose and sucrose were the different carbon sources used.

Analytical method

The degree of decolourization was measured spectrophotometrically and calculated from the adsorption values of the spectrum peaks obtained in comparison with the initial value: % decolourization = [(Absorbance of uninoculated broth-Absorbance of residual broth) / Absorbance of uninoculated broth] x 100.

Growth of the organisms in relation to decolourization was also determined at spectrum peak of 640 nm (Verhoven, 1996).

Statistics

The experiment was done in duplicate and data obtained were analysed for statistical differences using Duncan Multiple Range Test (DMRt).

RESULTS AND DISCUSSION

Screening / Preliminary decolourization experiment

The preliminary decolourization studies carried out with bacteria species isolated from the dye effluents, indicated that *B. cereus* performed best when compared to *M. acidophilus* and *S. faecalis* (Tables 1 - 3).

Effects of varying carbon sources in the fermentation medium on growth and decolourization of 2 reactive dyes by *B. cereus* under aerobic condition

There was 67.33% decolourization rate for cibacron black

Table 1. Preliminary decolourization of 5 dyes by *B. cereus* under anaerobic and aerobic condition in fermentation medium at 5 days of incubation.

Dyes	*B. cereus*			
	Anaerobic		Aerobic	
	Growth	%Decolourization	Growth	%Decolourization
CRP4B	0.64 b	65.33 b, c	0.34 b	68.00 b, c
CBPSG	0.82 b	88.33 a	0.50 b	50.67 e, c
RTB	0.30 b	35.00e, g	0.29 b	37.67 f, d
DY	0.15 b	32.00 i, e	0.25 b	13.00 i, l
ROH3R	0.23 b	23.00 f, l	0.24 b	20.67 f, l

Means not sharing a common letter in a column, indicates statistical difference using Duncan Multiple Range test at ($P < 0.01$).

Table 2. Preliminary decolourization of 5 dyes by *S. faecalis* under Anaerobic and Aerobic condition in fermentation medium at 5days of incubation.

Dyes	*S. faecalis*			
	Anaerobic		Aerobic	
	Growth	%Decolourization	Growth	%Decolourization
CRP4B	0.51 b	12.33 i, l	0.17 b	9.67 j, l
CBPSG	0.12 b	29.67 j, f	0.05 b	20.67 f, l
RTB	0.24 b	18.00 f, l	0.19 b	21.33 f, l
DY	0.17 b	13.00 i, l	0.23 b	11.67 i, l
ROH3R	0.34 b	18.33 f, l	0.17 b	12.07 i, l

Means not sharing a common letter in a column, indicates statistical difference using Duncan Multiple Range test at ($P < 0.01$).

Table 3. Preliminary decolourization of 5 dyes by *M. acidophilus* under Anaerobic and Aerobic condition in fermentation medium at 5 days of incubation.

Dyes	*M. acidophilus*			
	Anaerobic		Aerobic	
	Growth	%Decolourization	Growth	%Decolourization
CRP4B	0.14 b	9.33 j, l	0.12 b	5.67 l
CBPSG	0.44 b	54.67 b, d	0.44 b	49.17 e, c
RTB	0.30 b	37.67 f, d	15.00 a	27.00 f, j
DY	0.18 b	17.33 g, l	0.16 b	14.33 i, l
ROH3R	0.25 b	14.00 i, l	0.15 b	14.00 i, l

Means not sharing a common letter in a column, indicates statistical difference using Duncan Multiple Range test at ($P < 0.01$).

PSG when NH_4NO_3/Glucose was incorporated into the fermentation medium. This was followed by (37.33%) colour loss for NH_4NO_3/Lactose combination and (35.00 %) decolourization rate for NH_4NO_3/lactose. While in cibacron red P4B *B. cereus* grew best in NH_4NO_3/glucose and decolourizes best in NH_4NO_3/sucrose combination (81%). The growth of the bacteria was not significantly different in the fermentation medium (Table 4).

Effect of peptone and different carbon sources in the fermentation medium on growth and decolourization of two reactive dyes by *B. cereus* under aerobic condition

When peptone was used as nitrogen source and different carbon sources were combined, there was 67.67% colour loss by peptone/lactose in fermentation medium. This

Table 4. Effect of varying the carbon sources in the fermentation medium on growth and decolourization of two reactive dyes by *B. cereus* under aerobic condition.

MEDIA	Cibacron Black PSG		Cibacron Red P4B	
	Growth	%Decolourization	Growth	%Decolourization
NH_4NO_3/Glucose	1.82 f, e	67.33 b, h	1.80 e, g	62.67 j, c
NH_4NO_3/Sucrose	1.75 e, h	35.00 k, p	1.92 e	81.00 e, a
NH_4NO_3/Lactose	1.57 f, l	37.33 o, k	1.74 e, h	63.33 i, c

Means not sharing a common letter in a column, indicates statistical difference using Duncan Multiple Range test at (P < 0.01).

Table 5. Effect of peptone and different carbon sources in the fermentation medium on growth and decolourization of two reactive dyes by *B. cereus* under aerobic condition.

Media	Cibacron Black PSG		Cibacron Red P4B	
	Growth	%Decolourization	Growth	%Decolourization
PEPTONE/Glucose	0.47 m, r	60.00 e, j	1.52 g, i	73.33 a, f
PEPTONE/Sucrose	0.27 r	26.67 l, p	1.70 c, d	38.33 l, k
PEPTONE/Lactose	0.75 k	67.67 b, h	1.89 e	73.00 a, f

Means not sharing a common letter in a column, indicates statistical difference using Duncan Multiple Range test at (P < 0.01).

Table 6. Effect of yeast extract and different carbon sources in the fermentation medium on growth and decolourization of two reactive dyes by *B. cereus* under aerobic condition.

Media	Cibacron Black PSG		Cibacron Red P4B	
	Growth	%Decolourization	Growth	%Decolourization
Yeast Extract/Glucose	1.31 l	27.33 l, p	1.70 e, h	61.00 e, j
Yeast Extract/Sucrose	2.46 d, e	65.33 i, c	1.52 g, i	35.67 l, k
Yeast Extract/Lactose	2.54 d, c	75.67 e, a	1.52 g, l	48.00 l, g

Means not sharing a common letter in a column, indicates statistical difference using Duncan Multiple Range test at (P < 0.01).

was significantly higher than decolourization obtained from peptone/glucose (60%) and peptone/sucrose (26.67 %) in cibacron black PSG. *B. cereus* grew best in peptone/lactose combination for cibacron black PSG dye. Also for cibaron Red P4B, *B. cereus* decolourizes best when peptone/glucose was combined in the fermentation medium (73.33%), this was followed by peptone/lactose (73%) and the least decolourization rate was obtained in peptone/sucrose combination (38.33%). The bacterium grew best in peptone/lactose 1.89 and least in peptone/ glucose 1.52, (Table 5).

Effect of yeast extract and different carbon sources in the fermentation medium on growth and decolourization of two reactive dyes by *B. cereus* under aerobic condition

B. cereus, in yeast extract/lactose combination supported best the growth and decolourization of cibacron black PSG (75%) while yeast extract/glucose combination supported best and decolourizes best in cibacron Red P4B (64%) (Table 6).

Spectrophotometric analysis of decolourization

Absorption ratio at distinct wavelengths changed as time progressed. The sequential reduction in absorbance at dye's maximum wavelength was attributed to the reducetive cleavage of azo bond by viable or dead cells, thereby reducing chromophores and fused aromatic rings with the simultaneous formation of UV absorbing intermediates (Figures 1 and 2). The results obtained in this study indicated that bacterial species isolated from the dye-waste effluents have potential to decolourize dyes to varying degrees. Bacterial capable of dye decolourization have been reported. Oranusi and Ogugbue (2005) reported on degradation of sulphonated azo dyes by *Pseudomonas* sp. Kodam et al. (2005) also reported on

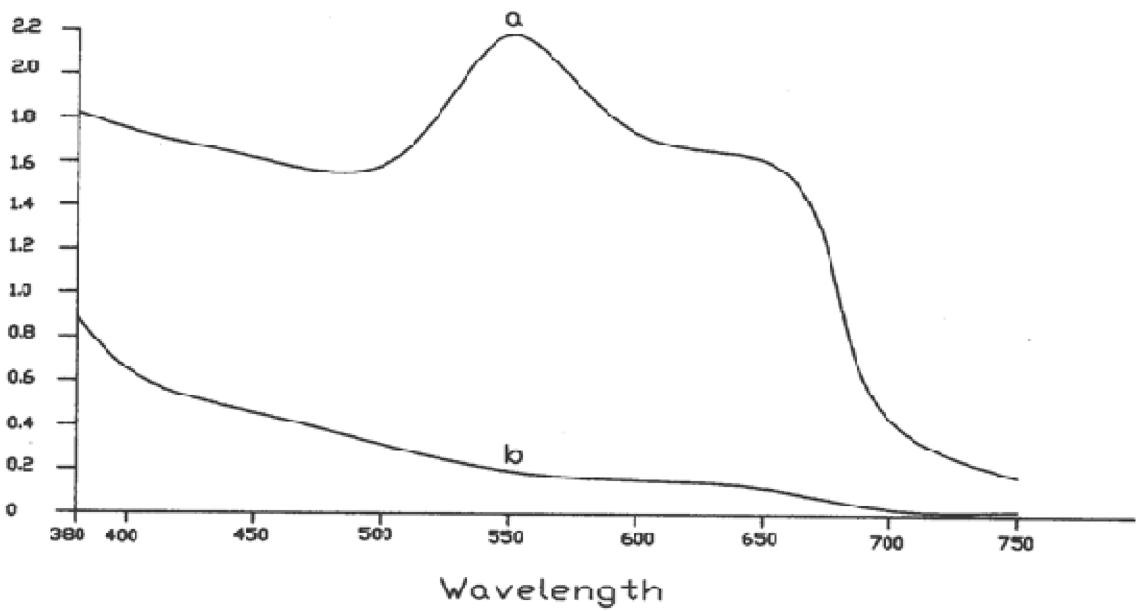

Figure 1. Spectrophotometric analysis of aerobic decolouration of cibacron black PSG by *B. cereus*. a) untreated dye at 0 day; b) treated dye after 5 days.

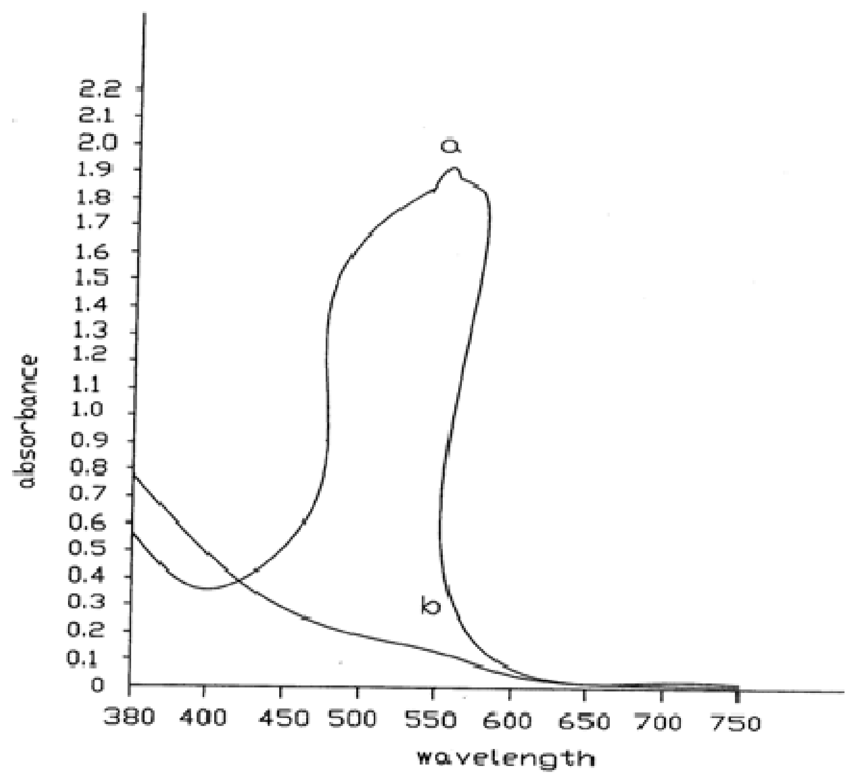

Figure 2. Spectrophotometric analysis of aerobic decolouration of cibacron red P4B by *B. cereus*. a) untreated dye at 0 day; b) treated dye after 5 days.

aerobic decolourization of reactive dyes. A similar result was obtained by Kumar et al. (2007), in decolourizing direct blue 15, using a bacterial consortium, where one member of the consortium is *Bacillus thuringiensis*. Dave and Dave (2009) also reported that *B. thuringiensis* exhibited excellent resistance and decolourization ability to AR-119 and other azo dyes. In this work, *B. cereus* had the highest decolourization rate of 81%. This was attained by *B. cereus* in the decolourization medium containing ammonium nitrate as nitrogen source and sucrose as carbon source, with cibacron red P4B dye. Generally, the percentage decolourization was better in supplemented media. This result agrees with the work of Padmavathy et al. (2003) in which simulated textile effluent was supplemented with starch and yeast extract to provide nutrients for biomass maintenance and to enhance biodegradation.

B. cereus performed well because they are nutritionally versatile and carries an efficient enzymatic system for the cleavage of azo bonds, which cause rapid decolourization of different azo dyes and thus they are able to biodegrade many natural and synthetic organic compounds.

This could be a consequence of natural adaptation of the organism as the sample from which the bacterial isolate was obtained were highly contaminated with dyes (Khera et al., 2005).

Although decolourization is a challenging process to both the textile industry and the wastewater treatment, the result of this findings and literature suggest a great potential for bacteria to be used to remove colour from dye waste. Interestingly, the bacteria species used in carrying out the decolourization in this study are isolated from the dye-industry waste effluents. Thus, biological processes that are simple, fast and economical can be adopted by textile and dyeing industries as an effective alternative for treating their wastewater.

However, further studies are needed to identify the biochemical processes involved in the decolourization of dyes.

Also, further examination of the effects of different nutrient sources on decolourization should be investigated. An important area to explore is the use of thermotolerant or thermophilic microorganisms in decolourization systems.

This would be of advantage as many textiles and other dye effluents are produced at relatively high temperatures.

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