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DECOLORIZATION AND DEGRADATION OF BLUE HEGN AND BLACK B DYES BY *Bacillus cereus*ISOLATED FROM THE TEXTILE DYE CONTAMINATED SOIL

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Abstract

The soil samples were collected from Jamaratextile Industry,Perundurai, Erode district. The collected samples were subjected to serial dilution and plating technique. The isolated microbial strains were identified based on the morphology, biochemical test and 16s rRNA Sequence. The soil isolates were subjected to textile dye Blue HEGN and Black B degradation based on the decolorizing potential. The most efficient strain was chosen after the immobilization studies. The 16S rRNA sequencing of the most efficient bacterial strain RR4 showed 99% similarity to *Bacillus cereus*. The analysis of degradation products by FTIR and UV- Vis analysis revealed disruption of dyes linkages and subsequent conversion of aromatic amines into simpler products. Bioremediation has proved to be very effective method in countering the textile dye pollution in an eco-friendly way. This approach creates a promising hope to remediate the environments polluted by textile azo dyes.

Key words: Textile Dye, *Bacillus cereus*, UV, FTIR and Carbon sources.

1. Introduction

The first human made synthetic dye, mauevin, was discovered in 1856 that took over the natural dye quickly. Since then, over 10,000 dyes have been generated worldwide with an annual production of over 7×10^5 metric tons. Synthetic dyes are widely used in textile, paper, food, color photography, paper printing, plastic, cosmetics, pharmaceutical, leather and toy industries (Zollinger, 1987 andCarliell*et al.*, 1995). Microorganisms like bacteria, actinomycetes, fungi and algae have been shown to degrade and biotransform azo dyes (Banat *et al.*, 1996). The azo dyes structures are reductively cleaved into colorless amines by several bacterial species. This behavior is often seen in aerobic bacteria that grow in the presence of azo compounds. (Mubarak Ali, 2011). By aerobic or anaerobic method bacterial decolorization of azo dyes is takes place (Pandey*et al*. 2007).

Textile effluent released from industries is a complex mixture of many polluting substances such as organo chlorine based pesticides, heavy metals, pigments and dyes (Saraswathy and Balakumar, 2009) and must be treated before discharged into environment because of their recalcitrant nature and potential toxicity to animals and human (Levine *et al.*, 1991; Hildenbrand*et al.*, 1999; Martins *et al.*, 2012). Dyes also obstruct light penetration and oxygen transfer that affects water bodies (Francisco*et al.*, 2009). Dyes are used in textile industry,

leather tanning industry, paper production, food technology, agricultural research, light-harvesting arrays, photo electrochemical cells, hair coloring and cosmetics. Moreover these compounds have been employed for the control of the efficacy of sewage and wastewater treatment, for the determination of specific surface area of activated sludge and for ground water tracing (Forgacs*et al.,* 2004; Hemapriya and Vijayanand, 2013).

In recent years a number of studies have focused on some microorganisms capable of degrading and absorbing dyes from wastewater. A wide variety of microorganisms are reported to be capable of decolonization of dyes (Chen *et al.,* 2003, Ponraj*et al.,* 2011 and Kumar *et al.*,2005). In this study bacterial strain, capable of decolorizing as well as textile dyes was isolated and the effect of various parameters. The degradation products were also characterized using FT-IR and UV-vis techniques.

2. MATERIAL AND MEHTODS

2.1 Collection of Sample

Soil samples were collected from contaminated sites of the Jamaratextile Industry, Perundurai, Erode district, Tamil Nadu.

2.2 Dyestuff and chemicals

The textile dyes, Disperse Blue HEGN and Black B were generous gift from Jamara textile industry,Perundurai, Erode, Tamil Nadu, India and used for this study without any further purification. All the other chemicals were used highest purity available and an analytical grade.

2.3 Isolation and screening of efficient textile dye decolorizing bacterial strains

Bacterial strains were isolated from contaminated soil samples were collected from Jamara textile industry,Perunduari, Erode. The soil samples were serially diluted by following the standard protocol and the dilution series 10^{-3} to 10^{-7} was plate on nutrient agar medium. All the plates were incubated at 37ºC for 24 hours. Culture was identified based on their morphology, color,and biochemical testand transferred aseptically into sterile agar slants for raising pure culture to perform further studies. Isolates were named as RR1 and RR8 respectively. All the isolated strainswere studied by inoculating them in Nutrient broth containing (g/l of dye containing effluent). The media peptone 10.0, yeast extract 2.0, Beef extract 2.0 and NaCl 5.0. The inoculated medium was incubated at 30ºC for 72 hrs under shaking and static conditions.

2.4 Identification of the isolate Strain

Identification of the isolated strain was performed by 16s rRNA sequence analysis. The taxonomical studies were carried out according to Bergeys manual. The 16s rRNA gene in this strain was amplified by 30 cycle of PCR using genomic DNA from strain RR4 as the template and two specific primes, 27F (5'AGAGTTTGATCMTGGCTCAG 3') and 1492 R (5'TACGGYTACCTTGTTACGACTT 3'). Each cycle was carried out at 94°C for 45 sec, at 45º C for 60 sec, and at 72ºC for 60 sec. DNA fragments were amplified about 1,400 bp in the case of bacteria. Sequencing was analyzed by Yazhas genomic lab.

2.5 Dye decolorization of the Nutrient broth

The dye decolorizing bacteria were screened using modified method of (Arunprasand and Bhaskara Rao 2010). Decolorization was detected by UV- Vis Spectrophotometer (Lambda 35) at respective λ_{max} using the supernatant from the liquid

culture media after centrifuge at 10000 rpm for 10 min in a refrigerated centrifuge.

2.6 Decolorization assay

Degradation assay was measured in the terms of percentage of decolorization using UV spectrophotometer. The percentage decolorization was calculated form the following formula.

% Decolorization = Initial OD – Final OD ×100 / Initial OD.

2.7 Different culture conditions

The decolorization of Blue HEGN and Black B were studied in different pH, temperature, carbon source and nitrogen source under shaking and static conditions.

2.8 FTIR analysis Method

FTIR analysis was carried out using Shimadzu 8400s spectrophotometer in the mid – infrared of 400 – 4000cm- with 16 scan speed.

3. Result and discussion

3.1 Absorption spectrum

The absorption spectra of two chosen dyes were studied in a double beam UV-Vis(Jacco 550) Spectrophotometer fig 1 and 4. From the optical density at 2nm bandwidth, the absorption maximum was determined and presented in Table 1.

3.2 Decolorization of shaking and static

Eight pure cultures were isolated from polluted soil ofJamara textile industry, Perunduari, Erode District, Tamil Nadu. All the eight strains were individually screened to find their potential in Blue HEGN and Black B (Table 2). Both dyes were decolorizedup to a 99% and 98% under shaking condition fig 2, 3. The static condition above dye decolorized to maximum of 99% and 97 figure 5, 6 by RR4 strain. Three strains were decolorized 44% Blue HEGN and Black B dyes. All the other isolates did not significant any changes in the above dyes. Sheth and Dave, 2009 reported 91.1% decolourization of Reactive Red BS. C. I.II by *Pseudomonas aeruginosa*. (Guo*et al*., 2008) reported that maximum azo acid dye removal rate of 5.3mg dye g cell-1 h-1. Further rise in inoculum size show no beneficial effects. Similar observations have been recorded by (Moosvi*et al.,* 2005).Decolorization of dyes may take place by adsorption (Aravindhan*et al.,* 2007).*B. cereus*has beenreported to decolorize different azo dyesfrom textile effluent. Modi *et al.,* 2010. Inspecting the cell mats also showed that microorganisms retained their natural color after decolorization of Blue HEGN and Black B. According to the literature (Knapp and Newby, 1995; Sani and Banerjee, 1999).

3.3 Identification of a Bacterial strain RR4

Isolated bacterial strain RR4 was spherical shaped and gram positive bacteria. Molecular studies revealed its characterization as DNA fragments are amplified about 1400bp nucleotide in length identified as *Bacillus cereus* and 16s ribosomal RNA gene partial sequences show similarity 99%.

3.4 Effect of different carbon sources

Different carbon and nitrogen source were used to optimize its respective medium. The decolorization of Blue HEGN and Black B by *Bacillus cereus* when glucose was used as carbon source was 94% and 93%. The percentage of decolorization of Blue HEGN and Black B with sucrose as carbon source was found to be 87% and 78% fig- 7. In another study (Ola *et al.,* 2010) have reported that maltose and peptone as the ideal carbon and nitrogen sources

respectively for efficient decolorization of Reactive Red 195 by *Bacillus cereus,* recording 97% reduction in color of the dye in the effluent. In another deems that glucose can inhibit the decolorizing activity (Chen *et al.,* 2003). The variability may be due to the different microbial characteristics involved. Our result showed that a certain concentration of carbon sources (such as glucose and sucrose) was necessary for the *Bacilus cereus* decolorizing process.

3.5 Effect of different Nitrogen sources

The effect of different nitrogen source was studied on decolorization of Blue HEGN and Black B dyes by *Bacillus cereus.* The decolorization percentage was 78% and 82% when beef extract used as nitrogen source.Blue HEGN and Black B decolorized up to be 72% and 83% respectively while peptone used as nitrogen source fig 8. Dye being deficient in carbon source, the biodegradation of dyes without any extra carbon source is very difficult (Nigam *et al.,* 1995 and*Ola et al*., 2010) have reported requirement of different carbon and nitrogen sources for maximum decolorization of the two dyes.

3.6 Different culture condition in pH growth of RR4 strains

The optimum pH is one of the important factors in the treatment of textile dye effluent by microorganism. pH ranging from 6 to 8 of the medium, the cell growth was tested at room temperature for 24 hours (fig 9). The growth of *Bacillus cereus* was recorded and slow growth rate was noticed at pH6 and better growth was reported at pH8±0.2. Previously reported that growth rate of this organism was slow and lag phase was long (Sheela *et al.,* 2006). Similar to most of the bacterial decolorization, none of the strain was able to decolorize the dye under aerobic (shaking) conditions (Chen *et al.,* 2003; Supaka*et al.,* 2004; Khehra*et al*., 2005; Moosvi*et al*., 2005).

3.7 Effect of temperature ondecolorizationof textile dyes

The temperature effect on decolorization rate was significant (Fig 10 and Table-3). Different temperature the Black B and Blue HENG dye concentration and pH 8 was fixed at 100$mg^{-1}$and pH 8 respectively.The most optimum temperature for decolroization was 40ºC shaking and static condition. However our observation on effect of temperature is further strongly supported by the investigation carried out by (Saratale*et al.,* 2010 and Bhatt Nikhil *et al*., 2012) reported that 37^{o}C temperaturegave maximum decolorization by bacterialconsortium. Dye decolorization can be strongly inhibited when a high concentration dyestuff was used to examine the poisonous effect of the dye on the degrading microorganisms (Kalme*et al.,* 2007; Khehra*et al*., 2005). Normally any microorganisms can show considerable improvement in growth of activity when kept at favorable temperature (Ondiso*et al.,* 2013).

3.8 FTIR analysis of Blue HEGN and Black B

The FTIR analysis was carried out to investigate the functional groups between parent dye and degraded metabolite. The results of FT-IR analysis of the Blue HEGN and Black B dyes obtained after decolorization showed various peaks (Fig. 11 and 14). The FT-IR spectra of effluent displays peaks at 3464, 2377, 1637 cm-1 and 3469, 2081, 1638 cm-1 for -OH and C=C stretching vibration, aromatic CH stretching vibration, C=O stretching vibration, respectively.

The results of FT-IR analysis of Blue HEGN dye obtained after shaking and static decolorization showed various peaks. The FT-IR spectra of effluent (Fig. 12 and 13) displays peaks at 3407, 2097,1053 and 3434, 2080, 1016 cm-1, for –OH stretching vibration, aromatic - CH stretching vibration, -C=C- stretching vibration, respectively. The results of FT-IR analysis of Black B dye obtained after shaking and static decolorization showed various peaks. The FT-IR spectra of effluent (Fig.15 and 16) displays peaks at 3439, 2079, 1638 and 3434, 2370, 1637 cm-1, for –OH stretching vibration, aromatic - CH stretching vibration, -C=C- stretching vibration, respectively. However, FT-IR analysis also confirms the biotransformation of the dyes. The previous report to FT-IR indicates the transformation in the structure of the molecule. Theses metabolites have been traced to amine compounds. Moreover, only a few bacterial strains were decolorizing type. The decolorization reaction was accompanied by the formation of a metabolite that showed up at 267 nm UV-Vis spectra (McMullan *et al.*, 2001).

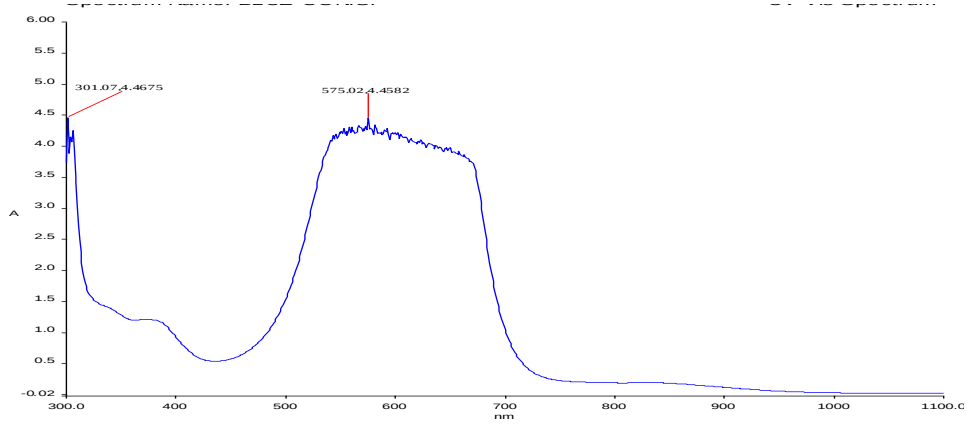

Fig 1 – Spectrum of Blue HEGN before decolorization

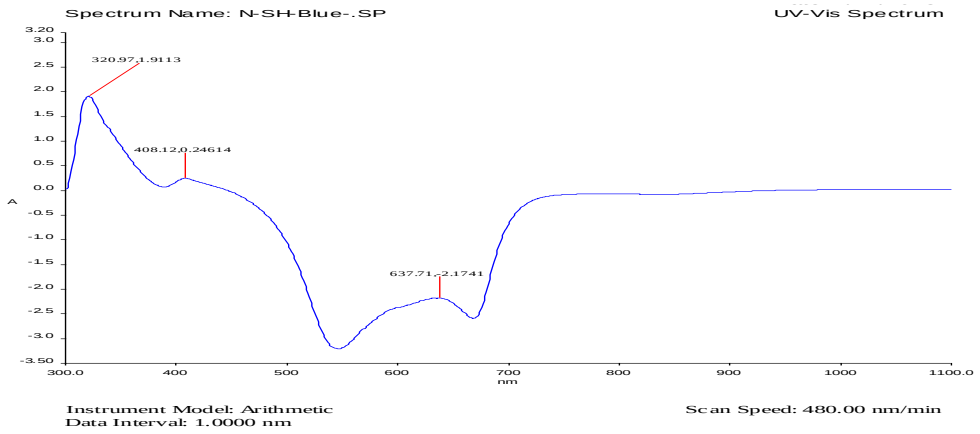

Fig 2 – Spectrum of Blue HEGN after (non –shaking) decolorization.

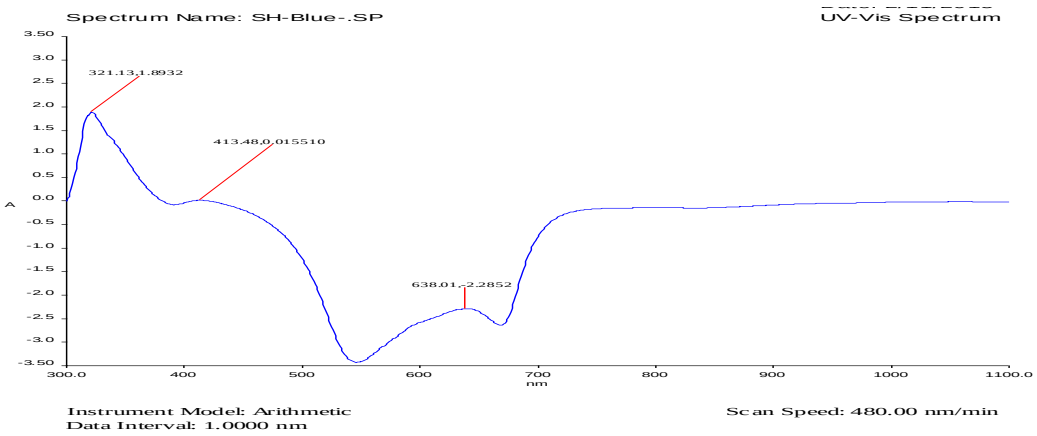

Fig 3 – Spectrum of Blue HEGN (Shaking) decolorization

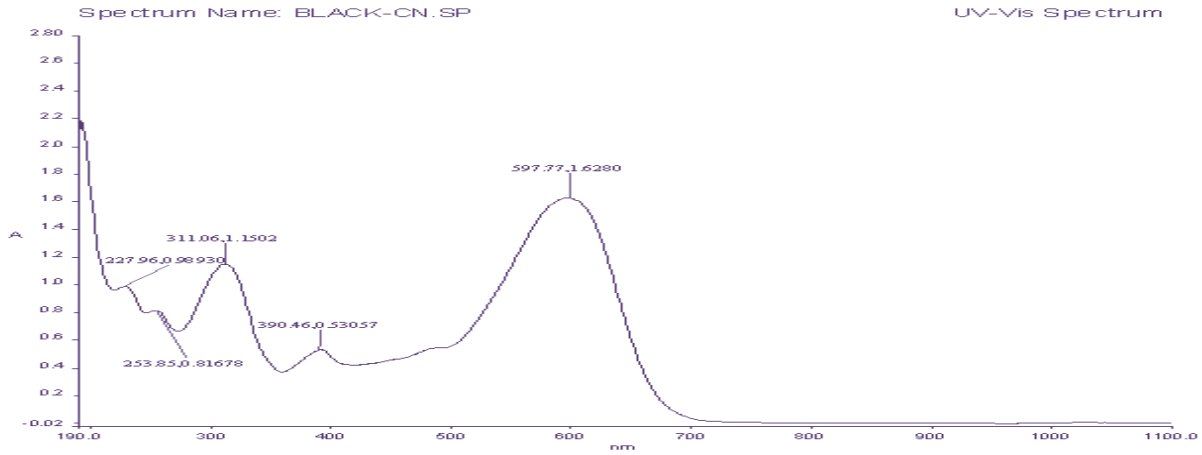

Fig 4 – Spectrum of Black B before decolorization

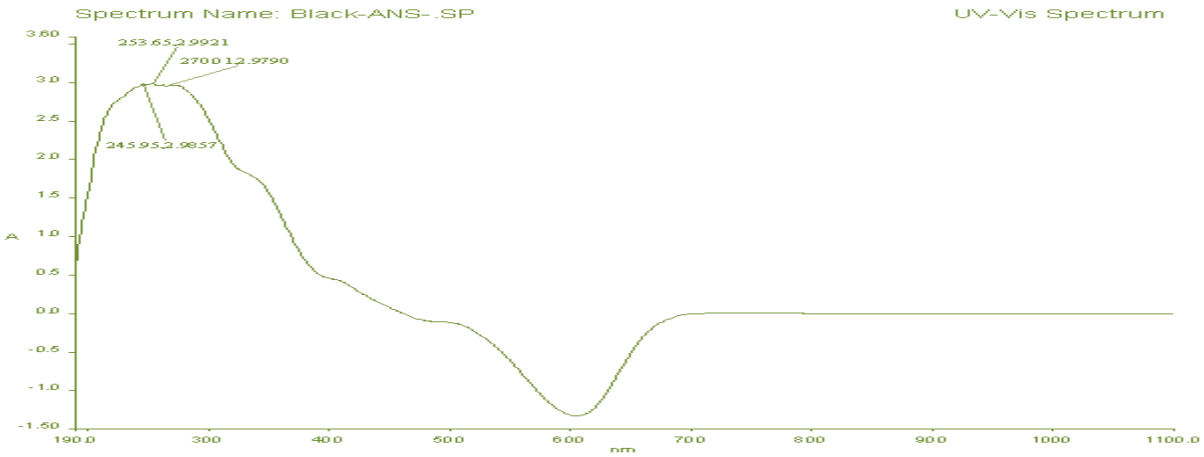

Fig 5 – Spectrum of Black B after (non-shaking) decolorization.

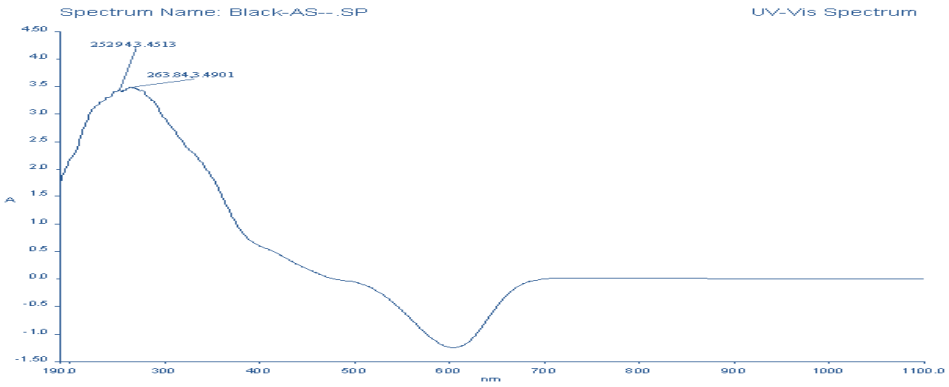

Fig 6 – Spectrum of Black B after (Shaking) decolorization

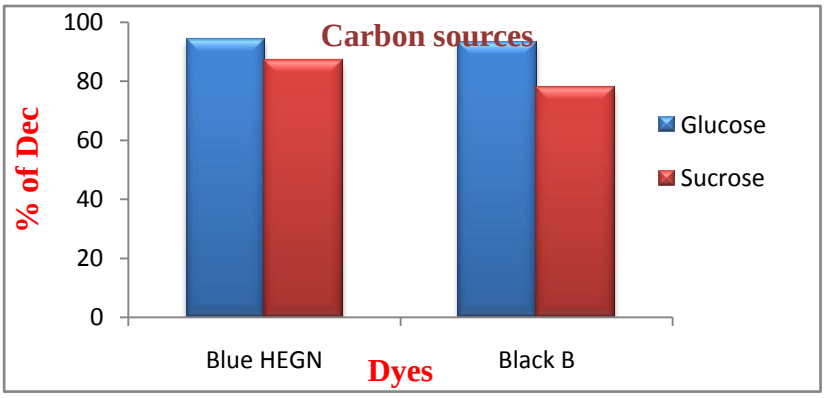

Fig - 7 Effect of Carbon sources

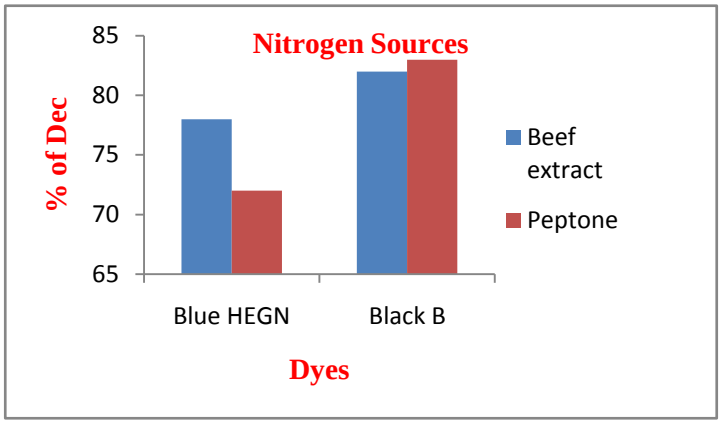

Fig – 8 Effect of Nitrogen sources

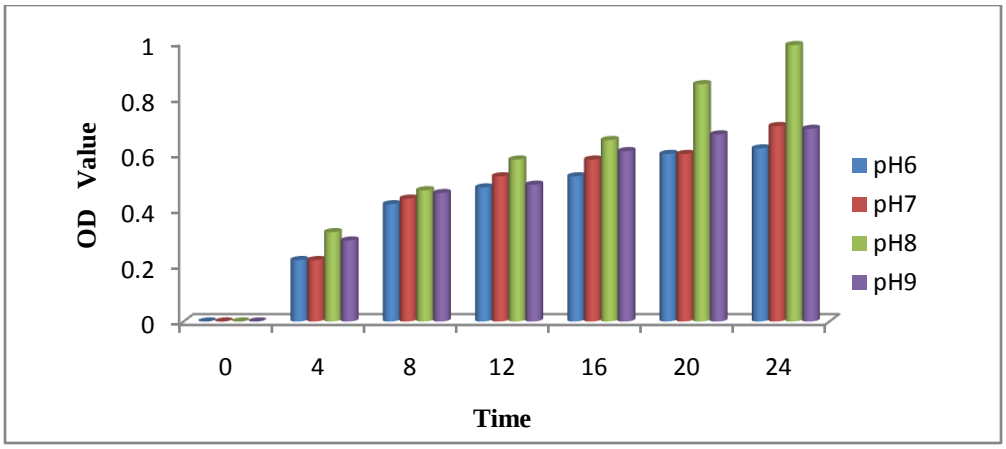

Fig- 9 RR4 Strain cell growth,

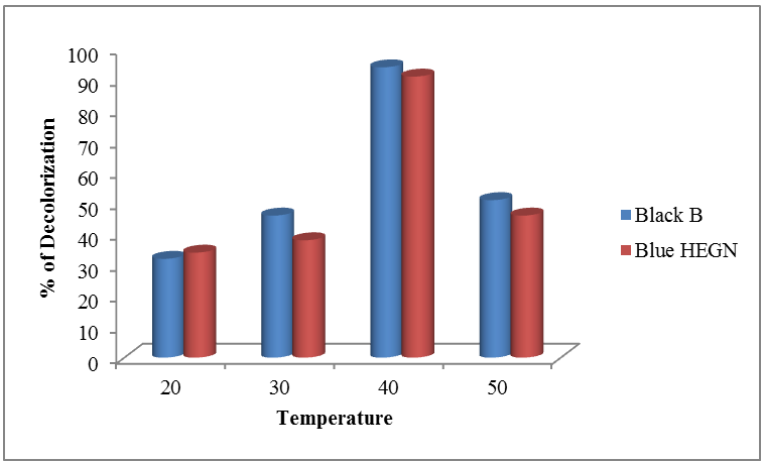

Fig – 10 Effect of Different Temperature

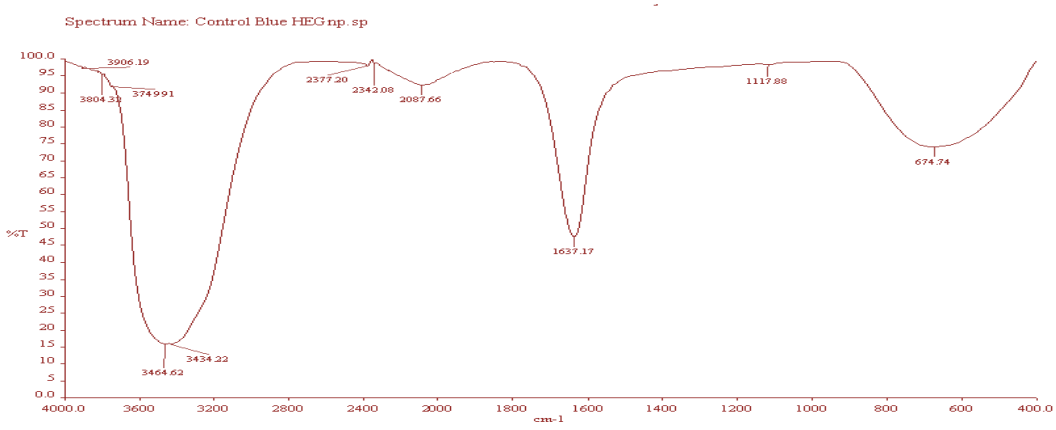

Fig 11 – FT-IR Spectrum of Blue HEGN before decolorization

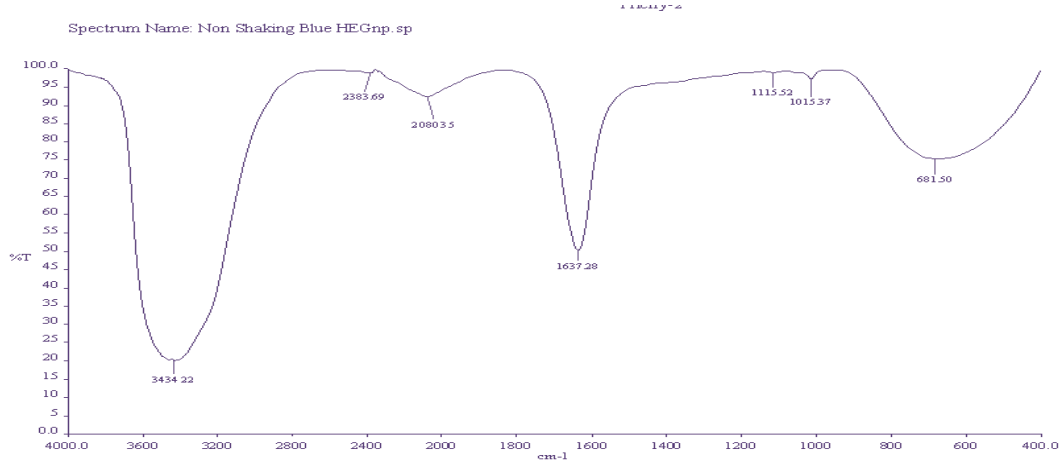

Fig 12 – FT-IR Spectrum of Blue HEGN after (non –shaking) decolorization.

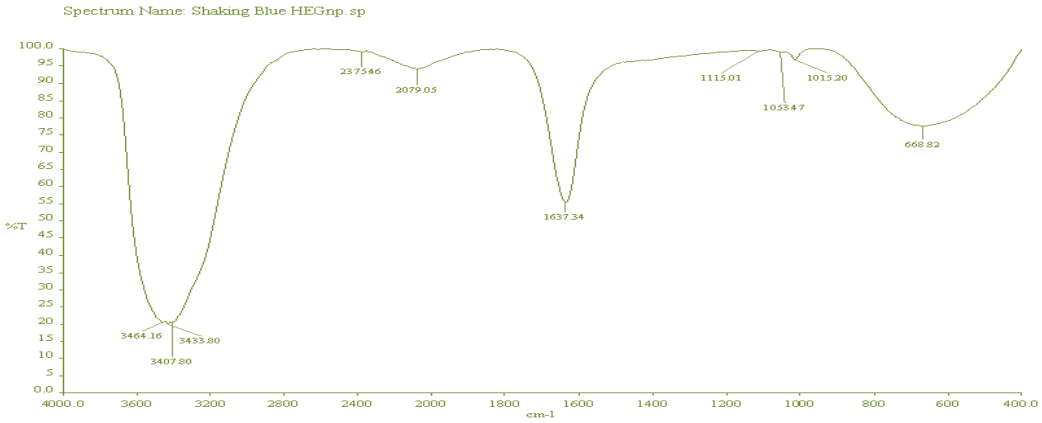

Fig 13 – FT-IR Spectrum of Blue HEGN (Shaking) decolorization

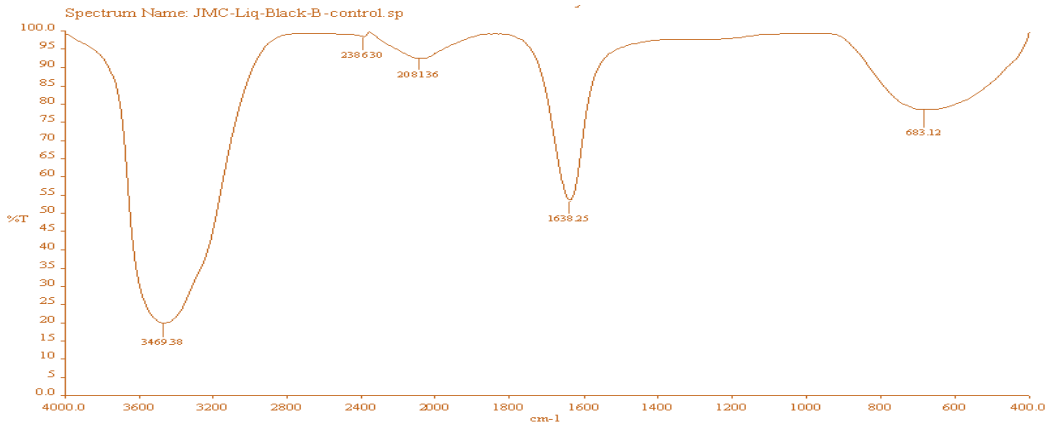

Fig 14 – FT-IR Spectrum of Black B before decolorization

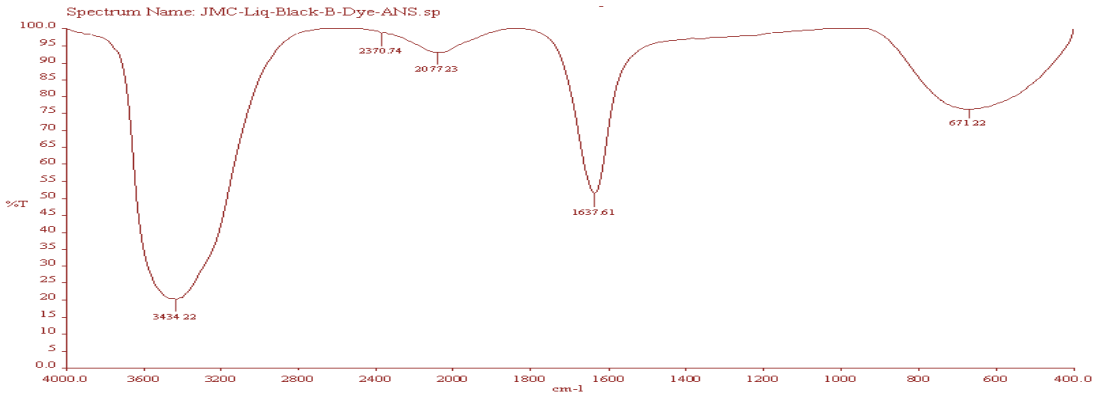

Fig 15 – FT-IR Spectrum of Black B after (non –shaking) decolorization.

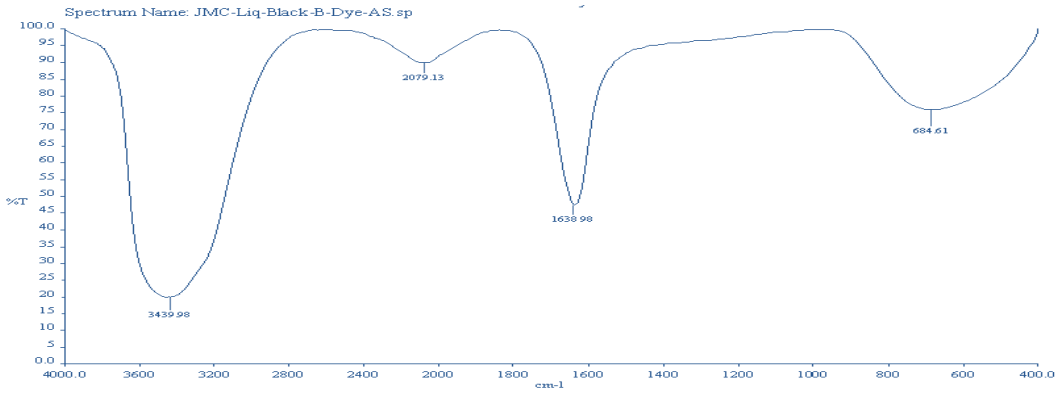

Fig 16 – FT-IR Spectrum of Black (Shaking) decolorization

S.No	Dyes	λ_{max} (nm)
1	Blue HEGN	575
2	Black B	597

Table 1 Showing absorption maxima (λ_{max}) of the dyes

S.No	Strain name	Blue HEGN	Black B
		%	%
1	RR1	42	41
2	RR2	35	34
3	RR3	ND	ND
4	RR4	99	97
5	RR5	ND	ND
6	RR6	45	42
7	RR7	ND	ND
8	RR8	ND	ND

Table 2 Decolorization of two dyes in nutrient broth by bacterial isolated (RR1 – RR8).

S.No	Temperature in ºC	% of Decolorization	
		Black – B	Blue HEGN
1	20	32	34
2	30	46	38
3	40	94	91
4	50	51	46

Table 3 Effect of temperature on the decolorization of Black-B and Blue HEGN.

4. Conclusions

The decolorization of textile dye was carried out by using *Bacilus cereus*under laboratory condition. The bacterial species used in carrying out the decolorization of Black- B and Bule HEGN in this study was isolated from the activated contained soil of Jamara textile industry, Erode.According to the result obtained in the present work, it can be concluded that the system employed can be suitable for use in dye decolorization, since it will able to operate with high efficiency to degrade different dyes in successive batches with no operational problems. In contrast to nitrogen sources and carbon sources showed inhibitory effects on the growth and the decolorization activity.*Bacillus cereus* showed good response in decolorizing the textile dyes. The result obtained in this study is very promising for the very effective. However, further work in needed to identify other genes responsible for this kind of textile reactive dyes decolorization.

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