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Biodecolourization of dye-contaminated textile effluents using *Bacillus cereus* N27

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Abstract

This study examined the potentials of *Bacillus cereus* N27 to decolourize textile effluents. The determined physicochemical parameters of the effluents were temperature, pH, total dissolved solids (TDS), total suspended solids (TSS), dissolved oxygen (DO), biological oxygen demand (BOD) and chemical oxygen demand (COD) using standard techniques. The *Bacillus cereus* N27 was isolated from textile effluent contaminated soil samples in Challawa, Kano state, Nigeria and were characterized and identified based on cultural, biochemical and molecular techniques. The *Bacillus cereus* N27 was used for the biodecolourization study. The 3 ml broth culture of *Bacillus cereus* N27 was inoculated into medium comprising 2 g of yeast extract, 2 g of glucose, 2.8 g of lactose broth in 50 ml of different concentrations of the effluent (0%, 5%, 10%, 30%, 50%, 70%, 90% and 100 %) and were incubated for 30 days at 37°C. The growth of the *Bacillus cereus* N27 was compared with corresponding McFarland standards. The rate of biodecolourization was determined using spectrophotometer at 600 nm. The effects of temperature (30°C, 35°C, 40°C, 45°C) and pH (4, 6, 8, and 10) on the decolourisation potential of the isolates was also determined. The results obtained for the physicochemical study were temperature (33.5°C), pH (6.10), BOD (0.13 mg/L), COD (123.5 mg/L). DO (0.162 mg/L), TSS (310 mg/L), TDS (465.5 mg/L). The optimum biodecolourization temperature was 35°C with 72.2% rate of biodecolourization after 30 days while pH 6 was optimum for the biodecolourization. The results obtained in the study suggest that *Bacillus cereus* can be used to develop an effective biological treatment system for the wastewaters contaminated with textile effluent.

Key words: Textile effluent, dye, biodecolourization, *Bacillus cereus*.

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Introduction

The textile industry plays an important role in the world economy as well as in our daily life, but at the same time, it consumes large quantities of water and generates large amounts of waste water. The composition of the chemical agents used in the textile sector are diverse in nature ranging from inorganic to organic molecules (Karthikeyan, and Anbusaravanan 2013).

Environmental problems such as appearance of colour in discharges from various textile industries, coupled with the increasing volume of water consumed by these textile industries, have made the treatment of effluent and its reuse increasingly attractive to the industry (Vilaseca et. al., 2010).

The release of a wide range of compounds from these industries creates disturbance to the ecosystem causing climatic changes, global warming, the depletion of the ozone layer as a result of photochemical oxidation, reduction in ground water levels both in the ground as well as the oceans (Verma et. al., 2013). Therefore, industrial effluents which contain azo dyes must be treated adequately before discharging into the environment to remove the toxicity of the dye from textile effluent (Karthikeyan, and Anbusaravanan, 2013).

Fundamental work has shown that a wide variety of microorganisms are capable of decolorizing a wide variety of dyes (Leena and Selva, 2011). Microorganisms belonging to different taxonomic groups of Algae, Bacteria, Fungi, and Actinomycetes have been reported for their ability to decompose azo dyes (Olukami et. al., 2006). The Bacteria used include *Escherichia coli*, *Bacillus* spp, *Clostridium* spp (McMullan, 2011). Improvement in the microorganism's ability to degrade pollutants could be achieved through modification of the environment or the organism (Beydilli et. al., 2012).

The enforcement of permitted level of physico chemical parameters and that of heavy metals levels in discharge textile effluent has not been followed strictly. According to Tambarlini et. al., 2012 some heavy metals found in these textile effluents have been found to be carcinogenic while some of the chemicals are poisonous to man and aquatic life depending on the duration of exposure and dose (World Health Organisation, WHO, 2012).

Environmental pollution caused by dye from textile industries is increasing at an alarming rate especially in the northern part of Nigeria where the textile industries are located. This has prompted this study on the biological control of dye pollutants by isolating the dye degrading microbes from contaminated soil. Biological decolorization has been investigated as a method to transform, degrade or mineralize dyes (Verma, 2013). Therefore, the aim of this study was to utilize *Bacillus cereus* N27 to decolorize dye-contaminated textile effluent.

Materials and Method

Collection of Samples

Samples were collected from the textile manufacturing industry in Challawa, Kano State The effluent samples were collected in a clean 25-liter polyethylene container and transported to the laboratory using ice pack chest stored in the refrigerator at about 4°C prior to analysis contaminated soil samples (approximately 20 g) from the three locations marked A, B, C were also collected using some clean, dry and sterile polythene bags along with sterile spatula. Soil samples were also collected from an adjacent site D which is 500 meters away serving as control.

Isolation of Bacillus cereus from soil sample

The 1 g of soil sample was added to 5 ml of nutrient broth and incubated at 35°C for 24 hours. After the incubation period, 0.1 ml of the supernatant of each tube containing suspension of soil and culture media was inoculated in nutrient agar plates by streaking at 30°C for 24 hours. After that, the plates were examined and the suspected colonies were stained by Gram staining method. The Gram-positive, rod-shaped, spore forming bacilli was selected for additional identification tests. Subsequent identification tests including citrate hydrolysis test, motility test, Indole production test, catalase test, nitrate reduction test, and production of H_2S was performed (Sriham and Reetha, 2015).

DNA Isolation, Amplification and Sequencing of Bacterial isolate.

The DNA of the organisms was extracted for molecular characterizations using 16S rRNA gene from the bacteria, chromosomal DNA was extracted with QIAamp DNA Mini Kit (Qiagen Inc, Germany). The16S rRNA gene was amplified using polymerase chain reaction (PCR) with the primer 27F (5^1- AGA GTT TGA TCM TGG CTC AG-$3^{1)}$ and reverse primer 1492R (5^1- ACC TTG TTA CGA CTT-3^1) from Macrogen, Korea. The PCR mixture will include 0.2 µg of template, 20 pmole of each primer, 10 mM of dNTP mixture, 5 µl of 10x pfu DNA polymerase buffer (Mg included) and 3 units of pfu DNA polymerase (Bioneer Co., Korea) in a 50 µl reaction volume. After which the phylogenetic analysis was performed to determine the evolutionary relationship of the strain with other validly published strain (Sriham and Reetha, 2015).

Biodecolourization Test

The biodecolourization test was carried out in accordance with a modification of the method reported by Walter et al., (2013) thus: 100 ml flask containing 50 ml of the effluent with 2 g of yeast extract and 2 g of glucose added as a co-substrate to augment the activity of the bacteria isolates that is serving as a booster for the organisms to perform optimally. The flask was sterilized by autoclaving at 121°C for 15 mins. The sterilized flask containing the effluent sample was inoculated with 3 ml inoculum of B. cereus N27 and compared with the corresponding Mcfarland standard to determine the size of inoculum and the test was done on 100% of effluent and other concentrations that included 90%, 70%, 50%, 30%, 10%, 5% and 0%. The rate of biodegradation was measured using a spectrophotometer at 540 nm (Walter et. al., 2013).

Determination of the Optimum Temperature for Biodecolourization

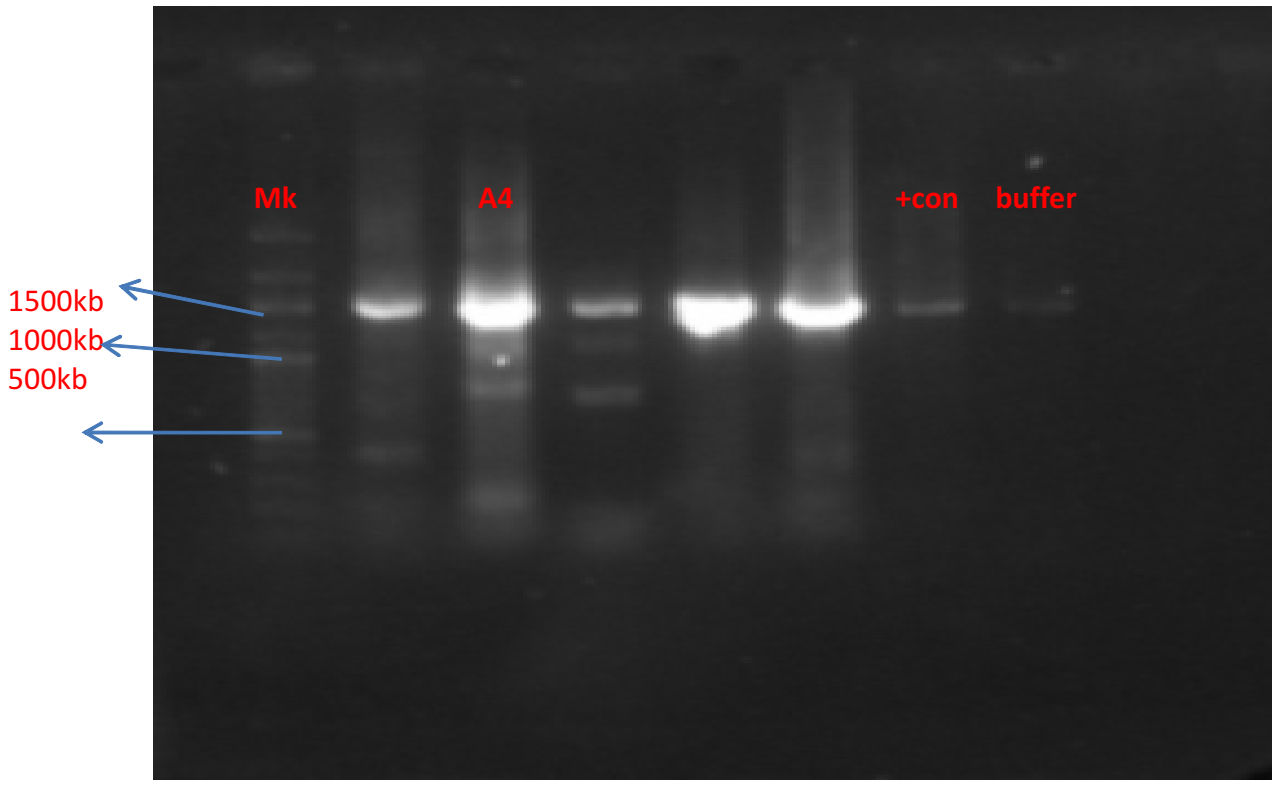
To determine the effects of temperature on the biodecolourization potential of the isolates, the procedure reported by Walter et al., (2013) was repeated with the following temperature ranges 30°C, 35°C, 40°C and 45°C in water bath. All other parameters such as available nutrient, oxygen were kept constant (Walter et. al., 2013).

Results

Table 1: Characterization of the isolates of *Pseudomonas* and *Bacillus* from dye contaminated soil

Test	Isolate A	Isolate B
Growth on CA	creamy coloured colony	ND
Growth on nutrient agar	ND	Gray dry colonies
Gram reaction	–	+
Motility	+	+
Catalase	ND	+
Oxidase	+	–
Nitrate	+	+
Lysin	+	ND
Ornithine	–	ND
Trehalose	ND	+
H_2S	–	ND
Glucose	–	ND
Mannitol	–	–
Mannose	ND	–
Xylose	+	–
ONPG	–	–
Indole	–	ND
Urease	–	ND
Citrate	–	–
TDA	+	–
Gelatine	–	ND
Malonate	+	ND
Inositol	–	–
Sorbitol	–	–
Rhamnose	–	–
Sucrose	–	–
Voges proskauer	ND	+
Galactose	ND	–
Arabinose	–	–
Cellebrose	ND	–
Adonitol	–	–
Raffinose	–	–
Salicin	–	+
Arginine	+	–
Growth at 25°C	ND	ND
Isolate	*Pseudomonas aeruginasa*	*Bacillus cereus*

Key: + = Positive; - = Negative; ND = Not Determined; ONPG = O-nitropHenyl-β-D-galactopyranoside; TDA = Tyrosine-D-arginine; CA= Centrimide Agar.



Key: kb=kilobase pares, Mk= DNA ladder 100bp, Cont= Control
A4= *Bacillus cereus* N27

Plate 1: The results of a PCR reaction using gel electrophoresis for *Bacillus cereus* N27

The result of a PCR reaction was visualized using gel electrophoresis (Plate 4). The result indicated that Isolate A4 DNA fragments form a band on the gel at location corresponding 1500 base pair (bp) of the DNA ladder.

The molecular characterisation of the isolate gave the following sequence: This gave a 98% identity to *Bacillus cereus* strain N27 16S ribosomal RNA gene,

GTTTGATCCCATGCTCAGGATGAACGCTGGCGGC
GTGCTCAATACATGCAAGTCGAGCCAAGTGGGGT
AAGAGCTTCTTCTTATGAAGTTAGCGGCGGACGG
GTGAGTAACACGTGGGTAACCTGCCCATAAGACT
GGGATAACTCCGGGAAACCGGGGCATATACCGGA
TAACATTTTGACCGCATGGTTCGAAATTGAAAGGC
GGCTTCGGCTGTCACTTATGGATGGACCCGCGTC
GCTTAGCTAGTTGGTGAGGTAACGGCTCACCAGG
GCAACGATGCGTAGCCGACCTGAGAGGGTGATCG
GCCACAATGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTAGGGAACTTCCGCAATGG
ACGAAAGGCTGACGGAGCAACGCCGCGTGAGTG
ATGAAGGCTTTCGGGCGTAAAACTTTGTTGTTAG
GGAAGAACAAGTGCTAGTTGAATAAGCTGGCACC
TTGACGGTACCTAACCAGAAAGCCACGGCTAACT
ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA
AGCGTTATCCGGAATTATTGGGCGTAAAGCGCGC
GCAGGTGGTTTCTTAAGTCTGATGTGAAACCCAC
GGCTCAACCGTGGAGGGTCATTGGAAACTGGGA
GACTTGAGTGCAGAAGAGGAAATGGAATTCCATG
TGTAGCGGTGAAATGCGTAGAGATATGGAGGAAC
ACCAGTGGCGAAGGGACTTTCTGGTCTGTAACTG
ACACTGAGGCGCGAAGCGTGGGGAGCAAACAGG
ATTAGATAACCCTGGTAGTCCACGCCGTAAACGAT
GAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAG
TGCCTGAAGTTAACGCATTAAGCACTCCGCCTGG
GGAGTACGGCCGCAAGGCTGAAACTCAAAGGATA
TTGACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGAAGCAACGCGAAGAACCTTACCA
GGTCTTGACATCTATCTGAAAACCCTAGAGATAGG
GCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGC
ATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCTTGATCTT
AGTTGCCATCATTAAGTTGGGCACTCTAAGGTGA
CTGCCGGTGACAAACCGGAGGAAGGTGGGGATG
ACGTCAAATCATCATGCCCCTTATGACCTGGGCTA
CACACGTGCTACAATGGACGGTACAAAGAGCTGC
AAGACCGCGAGGTGGAGCTAATCTCAAAAACCGT
TCTCAGTTCGGATTGTAGGCTGCAAATCGCCTACA
TGAAGCTTGAATCGCTAGAACTACCA

Biodecolourization abilities of the Isolates on the Textile Effluents

A selection process to explore the biodecolourization abilities of the Bacillus cereus N27 was carried out using Minimal basal medium containing the effluent at various percentages that is 1%, 1.5%, 2%, 2.5% and 5% respectively. *Bacillus cereus* N27 was found to possess the ability to biodecolourize textile effluent as it grew on the Minimal basal medium which contained the textile effluent. *Bacillus cereus* possessed the ability as it grew on Minimal basal medium containing 5% textile effluent. Its increased performance on higher concentration of textile effluent necessitated their selection in Table 2.

Table 2: Growth of Bacillus cereus on miminal Basal Medium containing Textile Effluents .

Isolate	1% v/v textile	1.5% v/v textile	2% v/v textile	2.5% v/v textile	5% v/v textile
Bc	+	+	+	+	+

Key: Bc= Bacillus cereus

Effects of Temperature on Biodecolourization of Textile Effluents

The results in Table 3 showed the effects of temperature on biodecolourization of the textile effluent. The biodecolourization activities increased with increase in temperature from 30°C to 35°C but later reduced at 40°C to 45°C. The highest activity of 65.2% at 35°C was at the different temperature ranges. *Bacillus cereus* N27 (Bc) showed the highest biodecolourization activity in Table 3.

Effects of pH on Biodecolourization of Textile Effluents

The activity of the Bacillus cereus N27 decreased with increase in pH values except in some instances at pH 6 and 8 on day 25. The *Bacillus cereus* N27 showed highest biodecolourization of 67.5% at pH6 and Table 4 also showed that higher pH were detrimental to the isolates because the biodecolourization activity began to decline at pH8. The result in Table 3 also showed that the activities of the isolates were affected at pH 8-10. At pH6 *Bacillus cereus* N27 had a percentage biodecolourization of 67.5%.

Table 3: Effect of varying temperature on the biodecolourization activites of textile effluent of *Bacillus cereus* N27 after 30 days

Days	Temperature(°C)	100%	90%	70%	50%	30%	10%
5	30	2.00^{b}	3.80^{a}	5.20^{b}	5.40^{c}	6.22^{c}	7.40^{c}
	35	4.00^{a}	3.40^{b}	5.00^{c}	10.00^{a}	10.40^{a}	13.20^{a}
	40	1.01^{c}	1.09^{c}	2.60^{d}	5.20d	7.00^{d}	10.30^{b}
	45	0.10^{d}	0.70^{d}	7.00^{a}	7.80^{b}	10.40^{a}	10.60^{a}
10	30	4.90^{b}	6.30^{a}	11.50^{b}	16.80^{b}	18.30^{b}	19.00^{c}
	35	5.20^{a}	4.39^{b}	11.00^{a}	17.00^{a}	21.00^{a}	20.90^{a}
	40	1.01^{d}	1.20^{d}	4.20^{d}	6.00^{d}	6.20^{d}	7.02^{d}
	45	1.20^{c}	2.00^{c}	7.10^{c}	9.00^{c}	12.00^{c}	11.30^{c}
15	30	8.00^{a}	9.30^{a}	17.10^{a}	21.20^{b}	22.00^{b}	22.40^{b}
	35	5.80^{b}	4.82^{b}	14.00^{b}	26.00^{a}	26.00a	28.00^{a}
	40	2.00^{d}	2.30^{c}	5.90^{d}	7.50^{d}	8.10^{d}	8.60^{d}
	45	2.70^{c}	2.20^{d}	9.80^{c}	10.70^{c}	13.70^{c}	14.90^{c}
20	30	11.40^{a}	13.10^{a}	18.00^{b}	23.30^{b}	23.70^{b}	24.90^{c}
	35	6.40^{b}	6.12^{b}	25.00^{a}	41.00^{a}	41.00^{a}	44.50^{a}
	40	4.10^{c}	4.00^{c}	5.60^{d}	7.00^{d}	7.30^{d}	8.70^{d}
	45	3.20^{d}	4.00^{d}	10.30^{c}	19.70^{c}	21.20^{c}	27.10^{b}
25	30	13.30^{a}	15.70^{a}	22.40^{b}	25.70^{b}	27.50^{c}	30.20^{c}
	35	7.20^{b}	6.90^{b}	44.00^{a}	48.00^{a}	48.00^{a}	60.70^{a}
	40	4.20^{c}	5.70^{c}	7.20^{d}	8.24^{d}	9.40^{d}	10.00^{d}
	45	3.60^{d}	5.10^{d}	14.40^{c}	22.60^{c}	35.00^{b}	38.00^{b}
30	30	14.90^{a}	17.50^{a}	30.60^{b}	34.50^{b}	37.90^{c}	41.20^{d}

Table 4: Effects of pH on biodecolourization of textile effluent

Days	Temperature (˚C)	100%	90%	70%	50%	30%	10%
5	30	2.00^{b}	3.80^{a}	5.20^{b}	5.40^{c}	6.22^{c}	7.40^{c}
	35	4.00^{a}	3.40^{b}	5.00^{c}	10.00^{a}	10.40^{a}	13.20^{a}
	40	1.01^{c}	1.09^{c}	2.60^{d}	5.20d	7.00^{d}	10.30^{b}
	45	0.10^{d}	0.70^{d}	7.00^{a}	7.80^{b}	10.40^{a}	10.60^{a}
10	30	4.90^{b}	6.30^{a}	11.50^{b}	16.80^{b}	18.30^{b}	19.00^{c}
	35	5.20^{a}	4.39^{b}	11.00^{a}	17.00^{a}	21.00^{a}	20.90^{a}
	40	1.01^{d}	1.20^{d}	4.20^{d}	6.00^{d}	6.20^{d}	7.02^{d}
	45	1.20^{c}	2.00^{c}	7.10^{c}	9.00^{c}	12.00^{c}	11.30^{c}
15	30	8.00^{a}	9.30^{a}	17.10^{a}	21.20^{b}	22.00^{b}	22.40^{b}
	35	5.80^{b}	4.82^{b}	14.00^{b}	26.00^{a}	26.00a	28.00^{a}
	40	2.00^{d}	2.30^{c}	5.90^{d}	7.50^{d}	8.10^{d}	8.60^{d}
	45	2.70^{c}	2.20^{d}	9.80^{c}	10.70^{c}	13.70^{c}	14.90^{c}
20	30	11.40^{a}	13.10^{a}	18.00^{b}	23.30^{b}	23.70^{b}	24.90^{c}
	35	6.40^{b}	6.12^{b}	25.00^{a}	41.00^{a}	41.00^{a}	44.50^{a}
	40	4.10^{c}	4.00^{c}	5.60^{d}	7.00^{d}	7.30^{d}	8.70^{d}
	45	3.20^{d}	4.00^{d}	10.30^{c}	19.70^{c}	21.20^{c}	27.10^{b}
25	30	13.30^{a}	15.70^{a}	22.40^{b}	25.70^{b}	27.50^{c}	30.20^{c}
	35	7.20^{b}	6.90^{b}	44.00^{a}	48.00^{a}	48.00^{a}	60.70^{a}
	40	4.20^{c}	5.70^{c}	7.20^{d}	8.24^{d}	9.40^{d}	10.00^{d}
	45	3.60^{d}	5.10^{d}	14.40^{c}	22.60^{c}	35.00^{b}	38.00^{b}
30	30	14.90^{a}	17.50^{a}	30.60^{b}	34.50^{b}	37.90^{c}	41.20^{d}

Discussion

The isolation of *Bacillus cereus* from the textile effluent contaminated soil confirms the reports of previous studies of Walter et. al, (2013); Sriram and Reetha (2015) that organism isolated from textile effluent is capable of utilizing dye. The role played by some bacterial species in the biodecolourization and decolourization of textile effluents has been reported by Sriram and Reetha (2015), Walter et. al. (2013), Karthikeyan and Anbusaravanan, (2013), Kalyanee et. al. (2010) and Ola et. al. (2011). Bacteria capable of decolourizing dyes from industrial effluent samples collected from waste water treatment sites contaminated by dyes have been isolated, screened and reported by Ndasi (2011). Idisi et. al. (2014) and Samuel et. al. (2013) isolated *Bacillus* species capable of degrading crude oil and textile effluents

The Results revealed that isolates of *Bacillus cereus* N27 achieved 67.5% a decolourization of textile effluent respectively. The results agreed with work carried out by Walter et. al. (2013). The ability of microorganisms to degrade textile effluent is as a result their constant metabolic activities. The isolates probably may have acquired the natural adaptive ability to survive in the presence of the textile effluent. The nitrogen, sulphate, and carbon found in the effluent medium are utilized by the microorganisms for their nutrition (Karthikeyan and Anbusaravanan, 2013).

The effects of temperature revealed a maximum percentage biodegradation of 64.1% at 35°C for *Bacillus cereus* as compared to the control which remained unchanged having a 0% biodegradation. This result is supported by the work carried out by Walter et. al. (2013) and Karthikeyan and Anbusaravanan (2013). Temperature has a significant effect on the biodecolourization of textile effluent by microorganisms (McMullan et. al., 2011). The *Bacillus* showed a higher performance at a higher temperature of 45°C. This could be as a result of a higher adaptive ability to high temperature which made the *Bacillus cereus* biodecolourization enzyme more resistant to temperature.

The effects of pH revealed that the maximum percentage of decolourization was 64.1% at pH6. This result is similar to the work of Karthikeyan and Anbusaravanan (2013) and supported the concept of Pandey (2008) that the growth of bacteria usually occurs at pH7. However, *Bacillus cereus* at pH10 result was slightly lower than at pH9 in biodecolourization. It is possible that pH10 was detrimental to the bacteria and caused the release of enzyme or redox mediators to cause dye reduction and the dye could also be reduced by alkaline hydrolysis (Kalyanee et. al., 2007). The control still remained unchanged even with the varying the pH. This implies that the presence of the microorganisms in the effluent may be responsible for the changes in colour of the effluent at the different pH.

The effects of co-substrates on the activities of the organisms indicated low biodecolourization activities of the organisms in the absence of glucose and yeast extracts and no form of activity in the control. This was in agreement with the work conducted by Walter et. al. (2013). This implies that the organisms could not metabolize the dye as sole source of carbon and energy to an appreciable level. An 81.2% biodecolourization was observed when the medium was supplemented with glucose and yeast extracts. Biodecolourization could further be increased and prolonged by supplementing the effluent medium with other cheaper effective carbon or energy sources such as sucrose (Karthikeyan and Anbusaravanan, 2013).

This work contradicts the previous work carried out by Murugalatha et al. (2010) that the maximum level exhibited by microorganisms to biodecolourize textile effluent is 90 days of incubation and the highest when compared to 14 days. The effects of incubation period indicated that the rate of biodecolourization rose up steadily over a period of 14 days while the control remained unchanged. Bacterial isolates require a shorter time for maximum efficiency (Singh et al., 2005). This potential for biodegradation is reiterated here (Husseiny, 2008). In this study, the rate of biodecolourization of the textile effluent gradually proceeded up to the 30th day.

The rate of decolourization at the initial days of exposure by the isolates to the textile effluent slowed down abruptly within the first 5 to 7 day of treatment while the control showed no signs of any activity of any microorganisms. The reason behind this slowed rate of biodecolourization could be as a result of the *Bacillus cereus* N27 still trying to adapt to the fresh environment different from their ecological niche. The rate of biodecolourization of textile effluent was rapid as the concentration of the effluent reduced from 100%, 90%, 70%, 50%, 30%, 10% and 0% (control) which displayed no sign of biodecolourization. This is in agreement with work done by Walter et. al. (2013).

Conclusion

The results of physico-chemical parameters obtained for textile effluents in this study indicated that the technique practiced by most textile industries in waste water treatment are inadequate in the removal of the pollution load generated in the production of textile materials. The activities of *Bacillus cereus* N27 resulted in the increased biodecolourization efficiency of textile effluents to an appreciable level within a shorter period of 30 days compared to 90 days. Therefore, *Bacillus cereus* N27 can be used to develop an effective biological treatment system for waste waters contaminated with dyes.

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