Journal of Applied Life Sciences International
5(2): 1-17, 2016; Article no.JALSI.24928
ISSN: 2394-1103

SCIENCEDOMAIN *international*
www.sciencedomain.org

Effect of Environmental Factors on Biodecolourization of Textile Effluents Using Mutagenised Strains of *Pseudomonas* and *Bacillus* Species

W. C. John[1] and N. C. J. Anyanwu[2*]

[1]Department of Pest Management Technology, Federal College of Forestry, Jos, Plateau State, Nigeria.
[2]Department of Biological Sciences, Faculty of Science and Technology, Bingham University, Karu, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author WCJ designed the study, wrote the protocol and wrote the first draft of the manuscript. Author NCJA managed the literature searches and analyses of the study performed the spectroscopy analysis. Authors WCJ and NCJA managed the experimental process and identified the species of microbes. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2016/24928
Editor(s):
(1) Shahira M. Ezzat, Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.
(2) Palanisamy Arulselvan, Institute of Bioscience, Universiti Putra Malaysia, Malaysia.
Reviewers:
(1) Fatih Kalyoncu, Celal Bayar University, Turkey.
(2) Anonymous, University of Guanajuato, Mexico.
(3) Noha Fathy Omar, Damietta University, Egypt.
(4) Khadijah Omar, Universiti Teknologi Mara, Malaysia.
(5) Selma Gomes Ferreira Leite, Federal University of Rio de Janeiro, Brazil.
(6) Anonymous, Hacettepe University, Turkey.
Complete Peer review History: http://sciencedomain.org/review-history/14552

Original Research Article

Received 9th February 2016
Accepted 27th April 2016
Published 10th May 2016

ABSTRACT

Aim: To determine the effects of varying environmental factors on textile effluents biodecolourization ability of mutagenised strains of *Bacillus* and *Pseudomonas* species isolated from dye contaminated soil.
Study Design: This research study was done using random sampling technique.

**Corresponding author: E-mail: anyanwunneomaconfidence@gmail.com;*

Place and Duration of Study: Challawa industrial Estate Kano; Department of microbiology, Ahmadu Bello University, between November 2014 and September 2015.
Materials and Methods: Samples were collected near a textile industry located at Challawa industrial Estate Kano. 20 liters of textile effluent were collected randomly at different points of stream where effluents are discharged and 20 g of dye contaminated soil was also collected at different location near the stream. *Bacillus cereus*, *Bacillus firmus*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* were isolated using standard procedure and identified using Microgen biochemical test kit. The *Bacillus* and *Pseudomonas* species were modified using Ultra-violet rays at 254 nm and Nitrous acid at different time interval (5, 10, 15, 20, 25 and 30 min). Biodecolorization ability of parent strains and wild strains and effects of varying environmental factors (pH, temperature, agitation, incubation period and co-substrates) was examined.
Results: The modified strains ($B8_{UV}30$) had higher textile effluent decolourization potentials of 60.30±0.02% than the parent strain ($B8_{WS}$) which showed 42.26±0.10% biodecolourization activities. The activities of the parent and modified strains were further compared based on effect of environmental factors (temperature, pH, incubation period, agitation and co-substrates). Examination of effects of varying environmental factor showed that the isolates exhibited different activities at different incubation conditions. At temperature of 35ºC, pH7, presence of co-substrate, incubation at 14 day and shaking, the isolates showed higher decolourization activities of 52.92±0.79, 58.82±1.02, 56.42±0.11, 66.16±1.16 and 58.38±0.01 respectively.
Conclusion: This study showed that mutation and environmental parameters used in this study has an effect on biodecolorization potentials of the strains and this could be employed in the biodecolourization of textile effluents to meet acceptable levels prior to discharge of textile effluents into the receiving environment.

Keywords: UV-irradiation; nitrous acid; textile effluent; biodecolourization; Pseudomonas; Bacillus; Azo dyes.

1. INTRODUCTION

Textile demand is steadily increasing worldwide following world population growth and stimulated by a growing gross domestic product (GDP) in many countries. Cotton and polyester account for 78% of the world's textile demand. Following this trend, reactive and disperse dyes are the most utilized dyes for colouring cellulosic fibres. Azo dyes that account for 60 – 80% of the dyes consumed in textile processing [1] are characterized by a typical double azo bond linkage (-N=N-), which is the most common chromophore of reactive dyes. The delivery of colour onto fabrics is not an efficient process and up to 40% of the dyes are lost during the dyeing process [2-4]. Dyeing, desizing and scouring are the major sources of water pollution in textile effluent. Textile industry is one of the oldest industries in Nigeria. In the last few years, environmental legislation about the appearance of colour in discharges, combined with increasing cost of water in the industrial sector has made treatment and re-use of dyeing effluents increasingly attractive to the industry [5,6]. Effluents from textiles and dye stuff industries discharge into water bodies is currently causing significant health concerns to environmental regulatory agencies. Government legislation is increasingly becoming more stringent especially in the more developed countries regarding the removal of dyes from industrial effluents. However, in developing countries like Nigeria, there is lack of legal enforcement of environmental legislation, mainly as a result of limited financial resources. This results in significant contamination of the environment by large industries. Protection and preservation of the environment in Nigeria is increasingly becoming a national concern. Textile and dyestuffs wastewaters are characterized by their highly visible colour, high chemical oxygen demand (COD), suspended solids and alkaline pH (9 – 11) [5,7] thus, effluent discharge from these industries into the environment is a major cause for concern. As dyes are designed to colour various substances and solutions indefinitely, there is a great potential for these dyes to accumulate in the environment as many of them are recalcitrant to normal bioremediation [8]. Several chemical and physical decolourization methods that are available include: adsorption, precipitation, coagulation/flocculation, oxidation, electrolysis and membrane extraction. These techniques are effective for colour removal but are energy

intensive and introduce chemicals which are not wanted in the first place. Dye wastewaters enter the environment from manufacturers and consumers (i.e. textile, leather and food industries) usually in the form of dispersion or a true solution [5,8,9] and often in the presence of other organic compounds originating from operational processes. Colour is the first contaminant to be recognized in textile wastewater and has to be removed before discharging into water bodies or onto land [5,10,11]. The presence of small amounts of dyes in water (even < 1 ppm) is highly visible and it affects the aesthetic merit, causes significant loss of luminosity and any increase in the temperature will greatly deplete the dissolved oxygen concentration in waste water. This results in subsequent alteration of the aquatic ecosystem [4]. The removal of colour from wastewater is often more important than the removal of soluble colourless organic substances which usually contribute to the major fraction of biochemical oxygen demand (BOD). Methods for the removal of BOD from most of these effluents are fairly well established [5,10,12]. On the other hand, textile wastewaters exhibit low BOD to COD ratios (< 0.1) indicating there difficulty to bioremediate or breakdown [13]. On the whole, textile wastewater is characterized by unfixed dyes, organic pollutants (much higher than regular domestic wastewater), large amounts of COD (organic compounds), high conductivity due to salts, high amounts of sulphide and heavy metals due to chlorinated bleaching agents and halogen, sulphur or heavy metal dyes [14]. Currently, much research has been focused on the biodegradation of the industrial effluents [15-17]. It mainly shows interest towards the pollution control using bacteria, fungi in combination with other biological methods involving plants [18,19]. *In situ* degradation of the effluent is a novel method under the biodegradation process. In this method, the microorganisms isolated from the site of pollution and the same microorganism can be used for the treatment of such site [5]. Improvement in the ability of microorganisms to degrade a pollutant could be achieved through modification of the environment or the organism. The organism can be modified through mutagenesis. This study was therefore, aimed at determining the effects of varying environmental factors on textile effluents biodecolourization ability of mutagenised strains of *Bacillus* and *Pseudomonas* species isolated from dye contaminated soil.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at the industrial area of Kano metropolis-Challawa. Kano is located in Kano State, it occupies a central position in Northern Nigeria. River Challawa (Lat 11° 52 m 41s N, Long 08° 28 m 08° 09s E) is 515 metres above sea level, originates from the Challawa Gorge dam in Challawa village and stretches to River Kano that empties into Lake Chad [5].

2.2 Sample Collection

Effluent samples and soil contaminated by untreated textile wastewater were collected in accordance with the methods reported by Roy et al. [20]. Samples were collected during dry season from November 2014 to April 2015. Effluent samples were collected at a point of discharge using clean 2-littre polyethylene container. Dye contaminated soil samples at different locations (1 km interval) from the stream into which effluent was discharged was collected from top 4 cm soil profile (approximately 20 g) using clean, dry and sterile polythene bags along with sterile spatula. The samples was labeled and transported to the laboratory using ice pack chest and stored in the refrigerator at about 4℃ prior to analysis.

2.3 Isolation of Dye Degrading *Bacillus* and *Pseudomonas* Species

Dye degrading bacteria were isolated from dye contaminated soil samples using the method reported by Saraswathi and Balakumar, [21]. Minimal basal medium which is composed of $(NH_4)_2SO_4$ 1.24 g, K_2HPO_4, 1.36 g, NaCl, 5.0 g, and $MgSO_4$, 0.1 g dissolved in 1000 ml distilled water, and trace elements $CuSO_4$(0.2 mg/l), $MnSO_4$ 1.1 mg/l, $FeSO_4$(0.14 mg/l) and $ZnSO_4$ (0.2 mg/l) and textile effluent (1, 1.5, 2, 2.5 and 5%) as a sole source of carbon was prepared. The medium (100 ml) in 250 ml Erlenmeyer flask was inoculated with 10 ml soil suspension (water diluted soil samples) in 100 ml Erlenmeyer flask and incubated at room temperature 37℃ on orbital shaker (150 rpm). After 48 hrs of incubation, 1.0 ml of the culture broth was appropriately diluted and plated on Nutrient Agar and King's B Agar using dilution 10^{-5} for the isolation of *Bacillus* and *Pseudomonas* species respectively. After incubation, the isolates were sub-cultured on nutrient agar and pure culture

stocks stored at 4℃ on nutrient agar slopes until needed.

2.4 Characterization of Colonies

Individual colonies were characterized using Microgen biochemical test-kits and used according to the manufacturer's instructions to identify the isolates to species level. Finally, comparison was made between the biochemical profiles obtained and tabulated characteristics in Bergey's Manual of systematic bacteriology to further identify the isolates.

2.5 Cultural Characteristic

The growth of the organisms on nutrient agar and centrimide agar was observed including pigment formation and odour of the colonies. Gram staining and motility test was also conducted using standard procedures.

2.6 Mutation with UV Irradiation at 254 nm

This was carried out by using a procedure reported by Idise [22]. The organisms were incubated in nutrient broth for 24 hours at 37℃ and their microbial count determined by calculating the colony forming unit. Ten milliliters of broth of each organism was aseptically transferred into separate sterile Petri dishes and exposed to UV irradiation at 6 cm from the source of UV light, the organisms were treated at intervals of 5, 10, 15, 20, 25 and 30 minutes in a dark room. The UV irradiated organisms were then transferred into a sterile twenty milliliter test tube in a dark room and treated with 0.2% (w/v) caffeine and allowed to stand at room temperature in the dark for 5 hours. The irradiated cells were then centrifuged at 1500 rpm for five minutes, re-suspended in normal saline and re-centrifuged and the supernatant used. The treated organism suspensions were incubated at 18℃ for 16 hours.

2.7 Mutation with Nitrous Acid

This was carried out by using the procedure reported by Iftikar et al. [23]. The bacterial growth was harvested from an overnight culture on nutrient broth and their microbial counts determined. The cell suspensions was mixed with the prepared nitrous acid (HNO_2) (0.2 M sodium nitrate in acetate buffer, pH 4.5) and treated at interval of 10, 15, 20, 25 and 30 min by incubating the mixture at 30℃. Treated cells were then centrifuged at 1500 rpm for three to five minutes, re-suspended in normal saline and re-centrifuged to remove traces of mutagen and supernatant discarded. The mutagenised cells were then plated on nutrient agar, incubated at 37℃ for 24 hours.

2.8 Screening for Amino Acid Requirement of Mutant

This was carried out using the procedure reported by Idise [22]. A minimal basal medium was used in the screening of mutated organisms. The medium was dispensed into sterile 20 ml test tube and inoculated with wild strains and selected mutant strains pre- grown on nutrient agar using sterile inoculating needle. Seven essential amino acids (histidine, leucine, lysine, methionine, phenylalanine, tryptophan and valine) were used due to their availability. To a set of seven test tubes, an amino acid were added and incubated with one test organism. A control was prepared containing the different amino acids without the test organism. The inoculated tubes were incubated at 37℃ for 24 hrs and observe for growth. Cloudy broths showed utilization of the test amino acid and were recorded as positive utilization of amino acid.

2.9 Biodecolourization Test

This was carried out in accordance with the method reported by Kalyanee, [24]. 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 was prepared. The pH of the medium was adjusted to 7±0.2 using phosphate buffer. The flask was sterilized by autoclaving for 15 mins at 121℃. The sterilized flask containing the effluent was inoculated with 3 ml inoculums ($3.6x10^9$CFUml) of each test micro-organism and incubated for 10 days at room temperature 37℃ on rotary shaker at the speed of 200 rpm. This was replicated thrice. Optical density (OD) at 560 nm was determined. After incubation, 10 ml of degraded effluent was centrifuged at 4000 rpm for 15 minutes. An un-inoculated flask containing textile effluent, which was sterilized by autoclaving served as control.

2.10 Decolourization Efficiency Calculation

Colour reduction % was determined using colorimeter at wavelength of 540 nm after 10

days intervals. The decolourization efficiency of the different isolates was calculated using the equation below.

$$\text{Decolourisation } (\%) = \frac{(I\text{-}F) \times 100}{I}$$

Where *I* is the initial absorbance and *F* is the absorbance of decolorized medium.

2.11 Effects of Environmental Factors on Biodecolourization Abilities of Strains

This was carried out by a modification of procedure reported by Kalyanee, [24]. Environmental condition such as pH, temperature, incubation time, agitation, presence and absence of co-substrate were varied and reported as follows:

2.11.1 Determination of effects of temperature

To determine the effects of temperature on the decolourization potential of the mutagenised isolates, modification of procedure reported by Kalyanee [24] was repeated with the following temperature ranges 30℃, 35℃, 40℃ and 45℃ in water bath. All other parameters were kept constant.

2.11.2 Determination of effects of pH

To determine the effects of pH on the decolourization potential of the mutagenised isolates, modification of procedure reported by Kalyanee, [24] was repeated with the following pH ranges 7,8,9 and 10. All other parameters were kept constant. 1N HCl buffered was used to adjust the pH towards acidity while 1N NaOH buffered was to adjust the pH towards alkalinity. Temperature maintained at room temperature 37℃ and all other parameters remains the same.

2.11.3 Determination of effects of incubation period

To determine the effects of incubation period on the decolourization potential of the mutagenised isolates, modification of procedure reported by Kalyanee [24] was repeated, but the incubation periods was extended to 14 days and reading taken at 2 days interval. All other parameters kept constant.

2.11.4 Determination of effect of agitation

To determine the effects of agitation and static condition on the decolourization potential of the mutagenised isolates, modification of procedure reported by Kalyanee [24] was repeated, the biodegradation test was carried out on a plane bench under static condition while under agitation the biodegradation test was carried out on rotary shaker at 150 rpm. All other parameters kept constant.

2.11.5 Determination of effects of co-substrate

To determine the effects of presence and absence of co-substrates on the decolourization potential of the mutagenised isolates, modification of procedure reported by Kalyanee [24] was repeated. In the absence of co-substrates, the biodegradation test was carried out without yeast extract and glucose. All other parameters kept constant.

2.12 Descriptive Statistic

Data were entered into Microsoft excel 2007 spread sheet and means and standard deviations were calculated.

3. RESULTS

3.1 Characterization of the Isolates

The isolates were identified as *Bacillus cereus*, *Bacillus firmus*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* as shown in Table 1 and 2. *Bacillus cereus* and *Bacillus firmus* showed positive Gram reaction, Oxidase, Motility and indole test. *Bacillus cereus* showed positive Arginine test and negative ONPG test. *Bacillus firmus* showed negative Arginine test and positive ONPG. *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* showed negative Gram reaction and positive Motility and Oxidase test. *Pseudomonas fluorescens* showed positive Citrate and urease test while *Pseudomonas aeruginosa* showed negative citrate and urease test.

3.2 Screening for Abilities of the Wild Strain Isolates to Grow in Different Concentrations of Textile Effluent

This is presented in Table 3. The organisms possessed the ability to grow in the presence of textile effluent as they metabolize dyes in the Minimal basal medium containing textile effluent leading to isolates multiplication. *Bacillus cereus* and *Pseudomonas aeruginosa* possessed a higher ability as they multiple in Minimal basal

medium containing 5% textile effluent while *Pseudomonas fluorescens* and *Bacillus firmus* grew in Minimal basal medium containing 2.5 and 2.0% textile effluent respectively.

3.3 Mutagenic Treatment of the Wild Strain Isolates

The effects of the mutagen treatment are presented in Fig 1 and 2. The effects of Ultraviolet (UV) irradiation (254 nm) on parent strains at different time 5, 10, 25, 20, 25 and 30 min, showed percentage survival that range from 3.04% *Bacillus cereus* ($B8_{UV}30$) to 56.15% *Pseudomonas fluorescens* ($P23_{UV}5$). The effect of nitrous acid (HNO_2 2.0 M) treatment after exposure of the parent strains ($B6_{WS}$, $B8_{WS}$, $P2_{WS}$ and $P23_{WS}$) at different time interval (10, 15, 20, 25, 30 min) presented in Fig. 2 showed percentage survival ranging from 10.37% *Pseudomonas fluorescens* ($P23_{NA}(10)$) to 58.76% *Bacillus cereus* (($B8_{NA}(10)$). The result in Figs. 1 and 2 showed that nitrous acid treatment of strains had a higher survival percentage and the result also showed that percentage survival is inversely proportional to time.

3.4 Screening for Amino Acid Requirement by Parent and Modified Strains

The requirement of essential amino acids – leucine, lysine, tryptophan, methionnine, histidine, valine and phenylalaine for growth by the parent and modified strains is shown in Table 4. It was observed that most of the organisms required all the tested amino acids for growth except $B8_{UV}30$, $P23_{NA}30$ and $P2_{UV}20$ which do not require histidine, valine and phenylalanine respectively. This showed the mutagenized strains having amino acid requirement different from their parents, hence, their selection for further analysis.

3.5 Biodecolourization of Textile Effluent

This is presented in Tables 5 and 6. It was observed that parent *Bacillus cereus* ($B8_{WS}$) performed better than the parent *Pseudomonas aeruginosa* ($P2_{WS}$), *Bacillus firmus* ($B6_{WS}$) and *Pseudomonas fluorescence* ($P23_{WS}$). The parent strains showed the following order of biodecolorization activities of 42.56±0.12, 40.43±0.62, 39.31±0.28 and 36.01±1.36% as shown in Table 5. The parents do not show any appreciable level of textile effluent biodecolourization. The mutagenised strains were better decolourizer than parent strains and also UV-irradiated strains performed better than the nitrous acid treated strains. *Bacillus cereus* ($B8_{UV}30$) irradiated at 30 mins and *Pseudomonas aeruginosa* ($P2_{UV}20$) irradiated at 20mins showed the highest percentage biodecolorization of 60.30±0.02% and 55.13±0.51% respectively while *Pseudomonas fluorescens* ($P23_{NA}30$) treated with nitrous acid at 30mins showed 53.09±0.37%. The results in Table 6 also showed that increase in time of treatment is directly proportional to percentage biodecolorization. The control samples showed no biodecolourization.

3.6 Effects of Temperature on Biodecolourization of Textile Effluent

The results in Fig. 3 showed the effects of temperature on textile effluent biodecolourization by the parent and mutant strains. The biodecolourization activities increased steadily with increase in temperature from 30℃ to 35℃ but later reduce at 40℃ to 45℃. Fig. 3 recorded the highest biodecolorization activities of 53.92±0.79% at 35℃ and the highest activity of 42.98±0.98% at 35℃ by parent. The mutant strains showed better biodecolourization than the parent at the different temperature range. *Bacillus cereus* ($B8_{UV}30$) and *Pseudomonas fluorescens* ($P23_{NA}30$) showed the highest biodecolourization activity. Mutant strains were more thermostable than the parent strains.

3.7 Effects of pH on Biodecolorization of Textile Effluent

The result of the effects of different pH values on biodecolourization activities are shown in Fig. 4. They activities of the parent and mutant strains decreases with increase in pH values except in some instances. The mutant strains showed highest biodecolourization of 58.82±1.02% while the parent strains showed highest biodecolourization of 43.20±0.8% at pH7. Fig. 4 also shows that higher pH were detrimental to the strains. The biodecolourization activity began to decline at pH8. Fig. 4. also shows that the activity of the mutant strains were affected at pH 8-10 but still did better than the parent strains. At pH10 *Bacillus cereus* ($B8_{UV}30$) has this highest percentage biodecolourization of 26.12±1.04% while parent *Bacillus cereus* ($B8_{WS}$) shows the highest biodecolourization of 7.21±0.76% among the parent strains.

3.8 Effects of Agitation on Biodecolourization of Textile Effluent

The results of the effects of agitation (shaking) and static condition on biodecolourization potential of the parent and modified strain is shown on the Fig. 5.The modified strains were better decolourizer on both static and shaking condition than the parent strains. The Fig. 5 result showed slight differences between the shaking and static condition. The highest biodecolourization activity (58.38±0.10) was shown by *Pseudomonas fluorescens* ($P23_{NA}30$) on state condition. The highest biodecolourization activities (54.83±1.05) on shaking condition were recorded by *Bacillus cereus* ($B8_{UV}30$). It's shown in Fig. 5 that both parent and mutant strains performed slightly better under static than shaking conditions.

3.9 Effects of Co-substrate on Biodecolorization of Textile Effluent

The results of the effects of co-substrate (glucose and yeast extract) on biodecolourization activity of both parent and mutant strains are shown in Fig. 6 The parent and mutant strains performed better in the presence of the co-substrate. The highest result in the absence of co- substrate was obtained by *Bacillus cereus* ($B8_{UV}30$) at percentage biodecolourization of 26.56±1.20 while the highest biodecolourization percentage of 56.42±0.11 in the presence of co-substrate was performed by the same organism. The parent strains showed the highest biodecolourization percentage of 13.92±0.69 and 43.74±0.54 in the absence and presence of co-subtrates respectively, which was achieved by *Bacillus cereus* ($B8_{WS}$) and *Pseudomonas aeruginosa* ($P2_{WS}$) respectively.

Table 1. Characterization of *Pseudomonas* isolates using microgen test kit

Test	After 12 h incubation	After 12 h incubation
Growth on CA	green coloured colonies	creamy coloured colony
Gram reaction	-	-
Motility	+	+
Oxidase	+	+
Nitrate	+	+
Lysin	+	+
Ornithine	-	-
H_2S	-	-
Glucose	+	-
Mannitol	-	-
Xylose	+	+
ONPG	-	-
Indole	-	-
Urease	+	-
VP	-	-
Citrate	+	-
TDA	+	+
Gelatine	-	-
Malonate	-	+
Inositol	-	-
Sorbitol	-	-
Rhemnos	-	-
Sucrose	-	-
Atabinose	+	-
Adonitol	-	-
Raffinos	-	-
Salicin	-	-
Arginine	+	+
Growth at 25^0C	+	ND
Isolate identified	*Pseudomonas fluorescens*	*Pseudomonas aeruginosa*

Key: + = Utilization; - = non-utilization; ND = Not Determined; ONPG = O-nitrophenyl-β-D-galactopyranoside; VP = Vogas proskauer; TDA = Tyrosine-D-arginine; CA= Centrimide Agar

3.10 Effects of Incubation Period on Biodecolourization of Textile Effluent

The results of the effects of incubation period on biodecolourization activity of both parent and mutant strain are shown in Fig. 7. These showed progressive increase in biodecolourization activities of the organisms as the days increase. The biodecolourization activities showed a decline at the day 10 with few progressing slowly to day 14. All the parent strains reached their peaks at day 10 with *Pseudomonas aeruginosa* ($P2_{WS}$) showing the highest activity of 51.57±0.70%. The mutant strains progressed steadily to day 14. *Bacillus cereus* ($B8_{UV}30$) showed the highest activity of 66.16±1.16% at day 14.

Table 2. Characterization of *Bacillus* isolates using microgen test kit

Test	After 12 h	After 24 h	After 12 h	After 24 h
Growth on nutrient agar	White dry colonies		Gray dry colonies	
Gram reaction	+	+	+	+
Catalase	+	+	+	+
Motility	+	+	+	+
Arabinose	-	-	-	-
Cellebrose	-	-	-	-
Inositol	-	-	-	-
Mannitol	-	-	-	-
Mannose	-	-	-	-
Raffinose	-	-	-	-
Rhamnose	-	-	-	-
Salicin	-	-	+	+
Sorbitol	-	-	-	-
Sucrose	-	-	-	-
Trehalose	-	-	+	+
Xylose	-	-	-	-
Adonitol	-	-	-	-
Galactose	-	-	-	-
MON	-	-	-	-
MDG	-	-	-	-
Inulin	-	-	-	-
Melezitose	-	-	-	-
Indole	ND	+	ND	-
ONPG	+	+	+	+
Arginine	-	-	-	-
Citrate	-	-	-	-
Voges proskauer	ND	+	ND	+
Nitrate	ND	+	ND	+
Isolates identified	*Bacillus firmus*		*Bacillus cereus*	

Key: + = Utilization; - = Non utilization; ND = Not determined ONPG = O-nitrophenyl-β-D-galactopyranoside. MON = Methyl-O-Mannoside; MDG = Methyl-D-Glucoside. 12/24h = time of incubation

Table 3. Growth of isolates on minimal basal medium containing textile effluent

Isolate	1% v/v textile	1.5% v/v textile	2% v/v textile	.5% v/v textile	5% v/v textile
$B8_{WS}$	+	+	+	+	+
$B6_{WS}$	+	+	+	-	-
$P2_{WS}$	+	+	+	+	+
$P23_{WS}$	+	+	+	+	-

Key: Ws = Wild strain; B8 = Bacillus cereus; P2 = Pseudomonas aeruginosa; B6 = Bacillus firmus; P23 = Pseudomonas fluorescens, + = growth, - = No growth

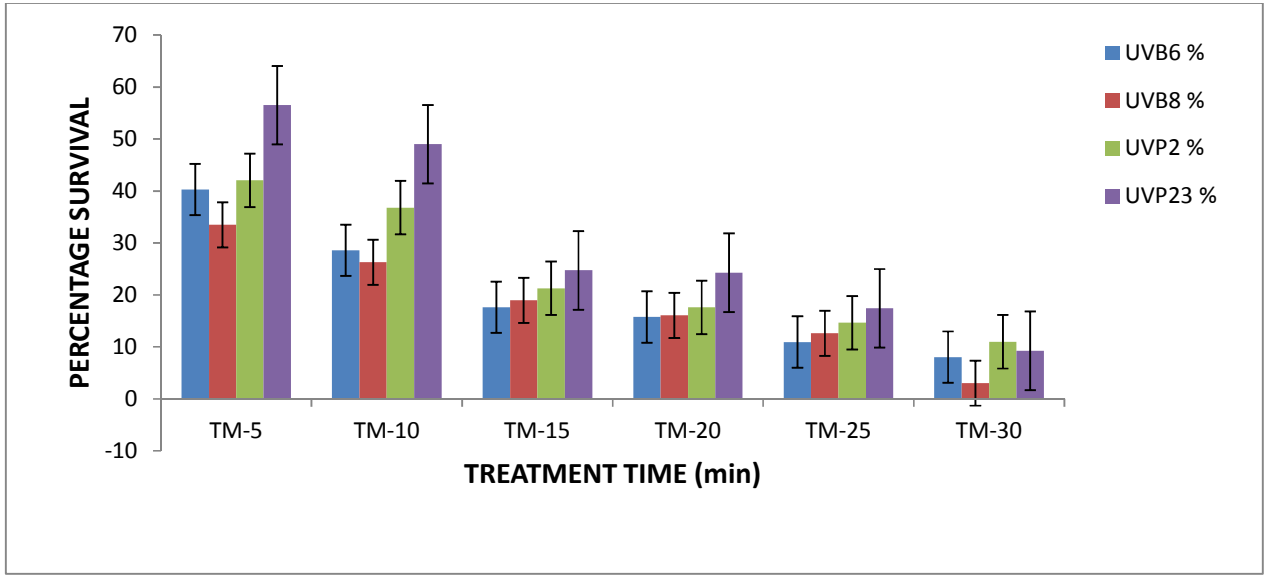

Fig. 1. Survival percentage of the UV-light exposed wild strains (ws) of *Pseudomonas* and *Bacillus* sp.
% = Percentage

Table 4. Screening for amino acid requirement of wild and mutagenised strains

Amino	$B8_{WS}$	$B8_{UV}30$	$B8_{NA}30$	$B6_{WS}$	$B6_{UV}20$	$B6_{NA}30$	$P2_{WS}$	$P2_{UV}20$	$P2_{NA}30$	$P23_{WS}$	$P23_{UV}25$	$P23_{NA}30$
Histidine	+	-	+	+	+	+	+	+	+	+	+	+
Lysine	+	+	+	+	+	+	+	+	+	+	+	+
Methionine	+	+	+	+	+	+	+	+	+	+	+	+
Phenylalanine	+	+	+	+	+	+	+	-	+	+	+	+
Valine	+	+	+	+	+	+	+	+	+	+	+	-
Leucine	+	+	+	+	+	+	+	+	+	+	+	+
Tryptophan	+	+	+	+	+	+	+	+	+	+	+	+

Key: *$B8_{UV}$ = UV-irradiated Bacillus cereus; $P2_{UV}$ = UV- irradiated Pseudomonas aeruginosa; $B6_{UV}$= UV- irradiated Bacillus firmus; $P23_{UV}$= UV- irradiated Pseudomonas fluorescens; $B8_{NA}$ = Nitrous acid treated Bacillus cereus; $P2_{NA}$ = Nitrous acid treated Pseudomonas aeruginosa; $B6_{NA}$= Nitrous acid treated Bacillus firmus; $P23_{NA}$= Nitrous acid treated Pseudomonas fluorescens; WS = Wild strains; + = Utilization ; - Non utilization*

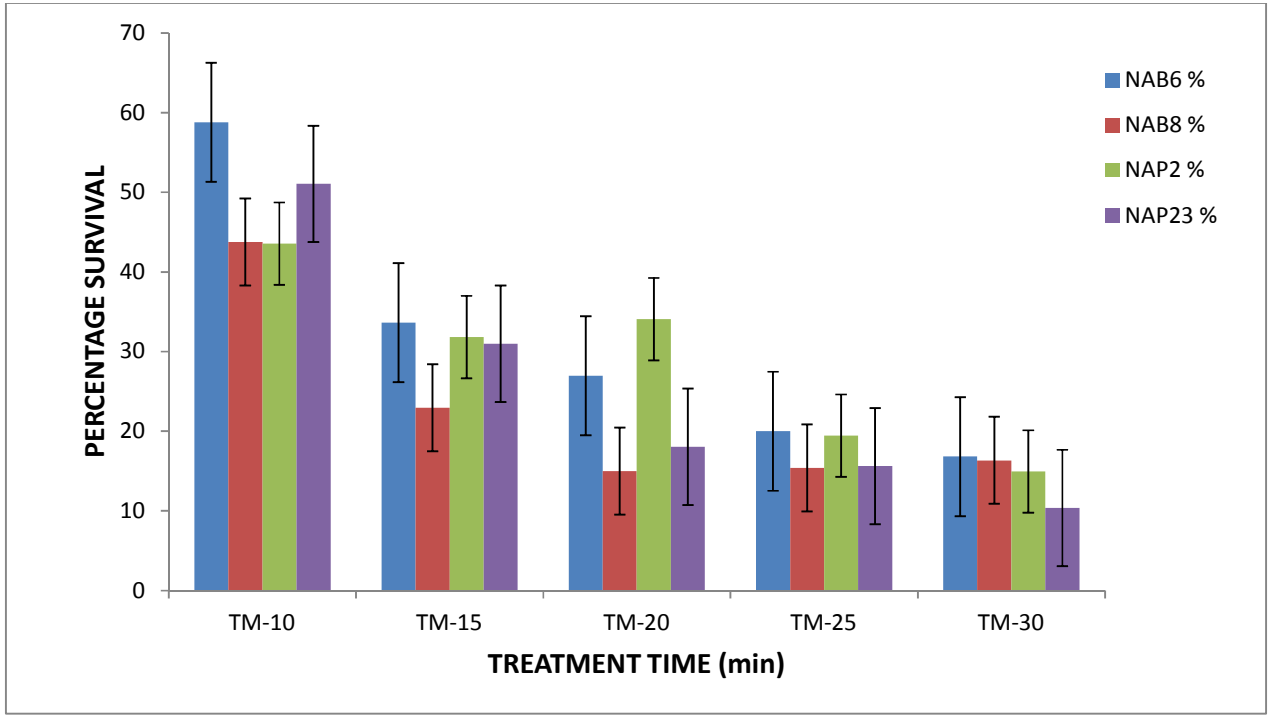

Fig. 2. Survival percentage of nitrous acid treated wild strains (ws) of *Pseudomonas* and *Bacillus* sp
% = Percentage

Table 5. Mean percentage biodecolourization performance by wild strains of the isolates

Strains	% biodecolourization of textile effluents
$B8_{WS}$	42.56±0.12
$P2_{WS}$	40.43±0.62
$B6_{WS}$	39.31±0.28
$P23_{WS}$	36.01±1.36

Key: Ws = Wild strain; B8 = Bacillus cereus; P2 = Pseudomonas aeruginosa; B6 = Bacillus firmus; P23 = Pseudomonas fluorescens.

4. DISCUSSION

The role of some bacterial species in the biodecolourization of textile effluents has been reported [24-26]. Chen et al. [27] also reported isolation and screening of bacteria capable of decolorizing various dye from industrial effluent samples collected from wastewater treatment site contaminated by dyes. The isolates *Bacillus* species and *Pseudomonas* species were susceptible to mutation with UV-light irradiation and nitrous acid treatment. Hence they were UV-light and nitrous acid sensitive. The treatment of the wild strains with ultraviolet irradiation and the nitrous acid treatment indicated that the increase in duration of the exposure of the organisms to the mutagen is directly proportion to the increase in rate of biodecolourization activities of the organisms. Mutant strains $B8_{UV}30$ obtained after 30 minutes irradiation gave highest biodegradation of 60.30±0.02%. This study disagrees with the previous work by Ado et al. [28] which state that it is not directly proportional.

Table 6. Mean percentage biodecolourization performance by mutagenized strains

Strains	% biodecolourization of textile effluent
$B8_{UV}30$	60.30±0.02
$P2_{UV}20$	55.13±0.51
$P23_{NA}30$	53.09±0.37

Keys: $B8_{UV}30$ = UV-irradiated Bacillus cereus, $P2_{UV}20$ = UV-irradiated Pseudomonas aeruginosa, $P23_{NA}30$ = Nitrous acid treated Pseudomonas fluoresens. 30 and 20 = Time of exposure to mutagens, ± = Standard error

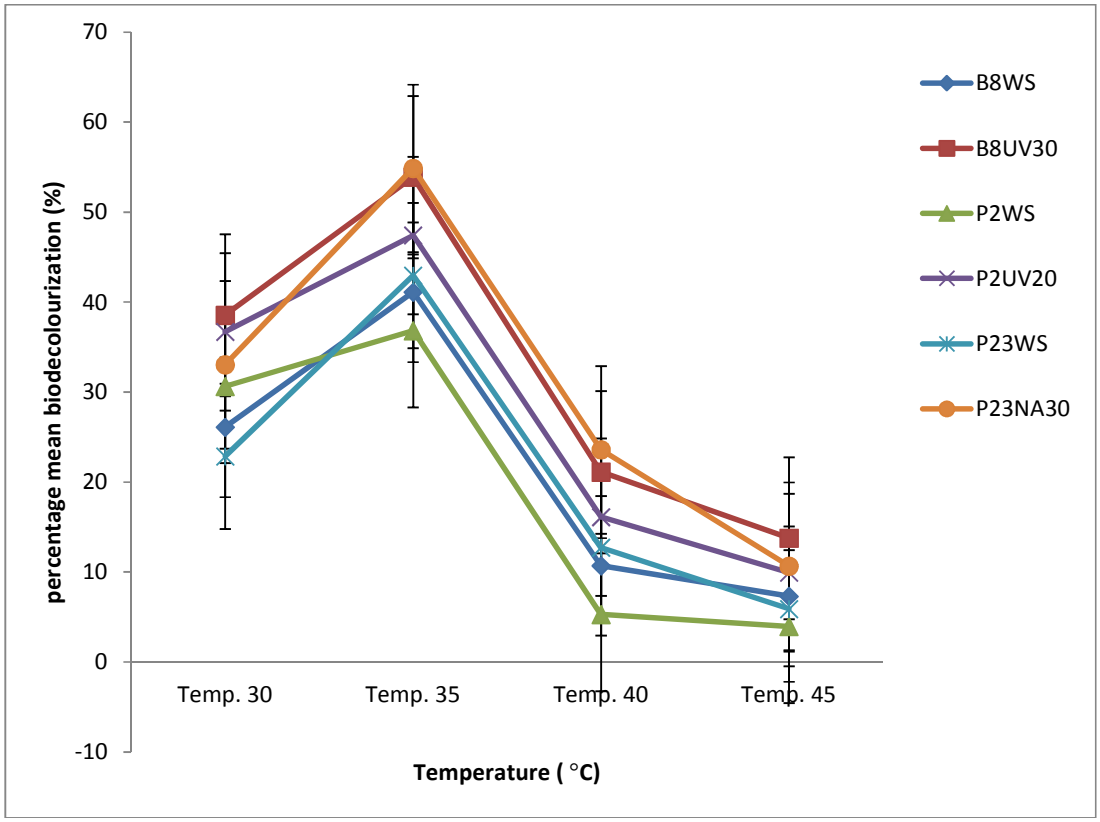

Fig. 3. The effects of temperature on biodecolourization of textile effluent

***Key**: $B8_{UV}$ = UV-irradiated Bacillus cereus; $P2_{UV}$ = UV- irradiated Pseudomonas aeruginosa; $P23_{NA}$= Nitrous acid treated Pseudomonas fluorescens; WS = Wild strains; Time of treatments = (20 and 30 minutes)*

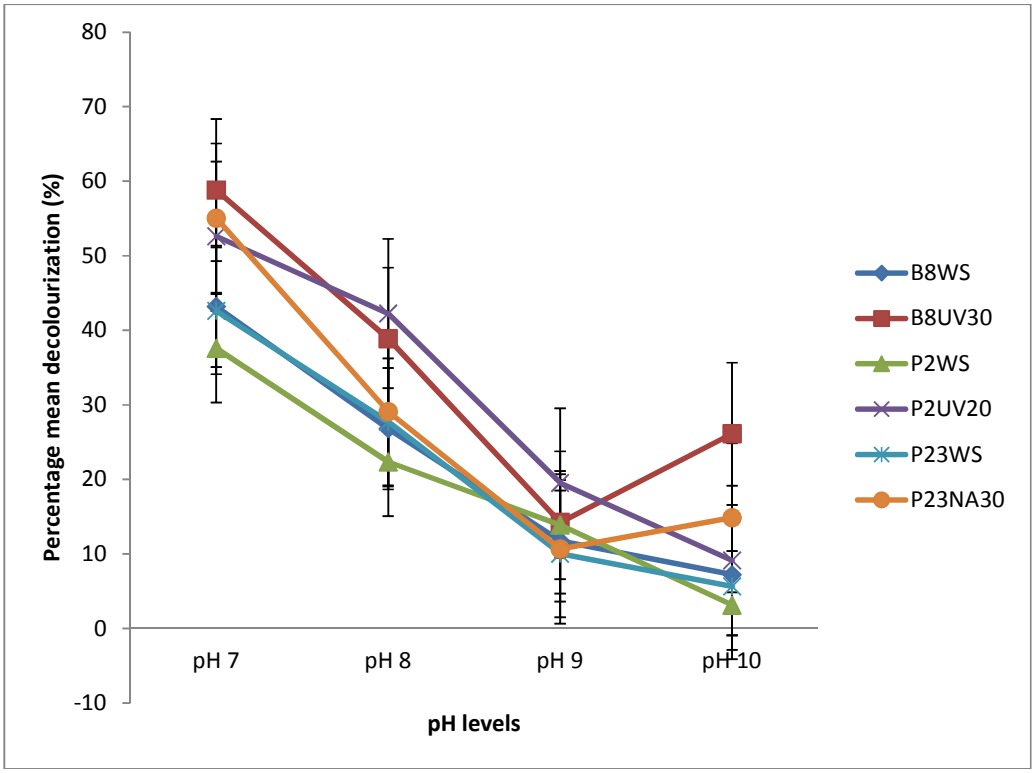

Fig. 4. The effects of pH on biodecolourization of textile effluent

Key: *$B8_{UV}$ = UV-irradiated Bacillus cereus; $P2_{UV}$ = UV- irradiated Pseudomonas aeruginosa; $P23_{NA}$= Nitrous acid treated Pseudomonas fluorescens; WS = Wild strains; Time of treatments = (20 and 30 minutes)*

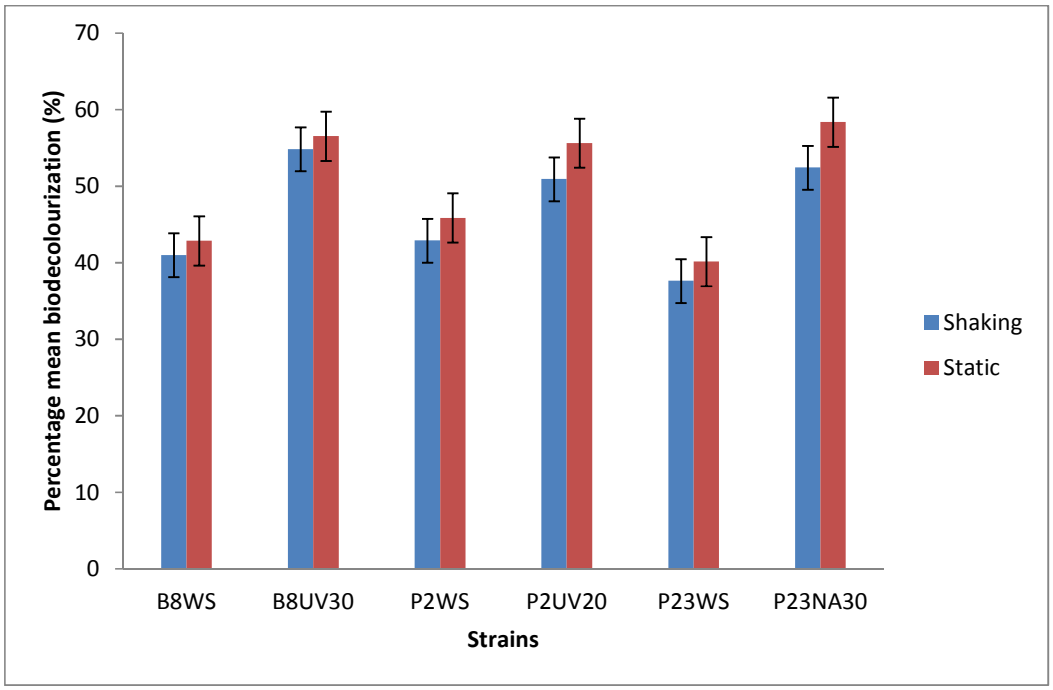

Fig. 5. The effects of agitation on biodecolourization of textile effluent

Key: *$B8_{UV}$ = UV-irradiated Bacillus cereus; $P2_{UV}$ = UV- irradiated Pseudomonas aeruginosa; $P23_{NA}$= Nitrous acid treated Pseudomonas fluorescens; WS = Wild strains; Time of treatments = (20 and 30 minutes).*

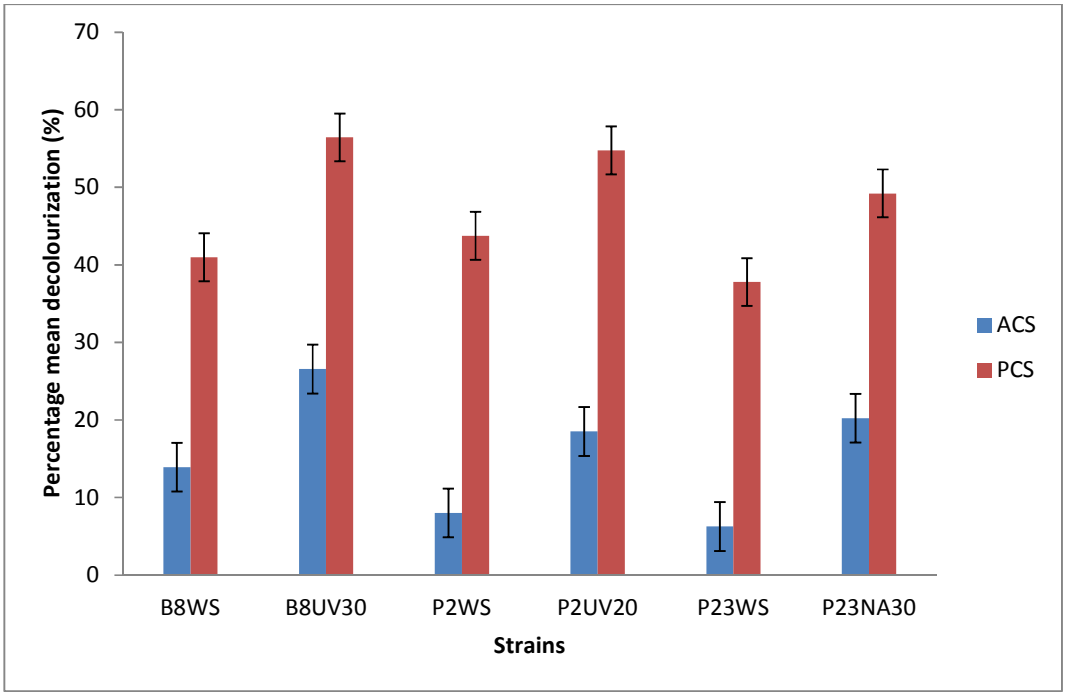

Fig. 6. The effects of co-substrate on biodecolourization of textile effluent

Key: $B8_{UV}$ = UV-irradiated Bacillus cereus; $P2_{UV}$ = UV- irradiated Pseudomonas aeruginosa; $P23_{NA}$= Nitrous acid treated Pseudomonas fluorescens; WS = Wild strains; Time of treatments = (20 and 30 minutes); ACS = Absence of co-substrates (glucose and yeast extract); PCS = Presence of co-substrates (glucose and yeast extract)

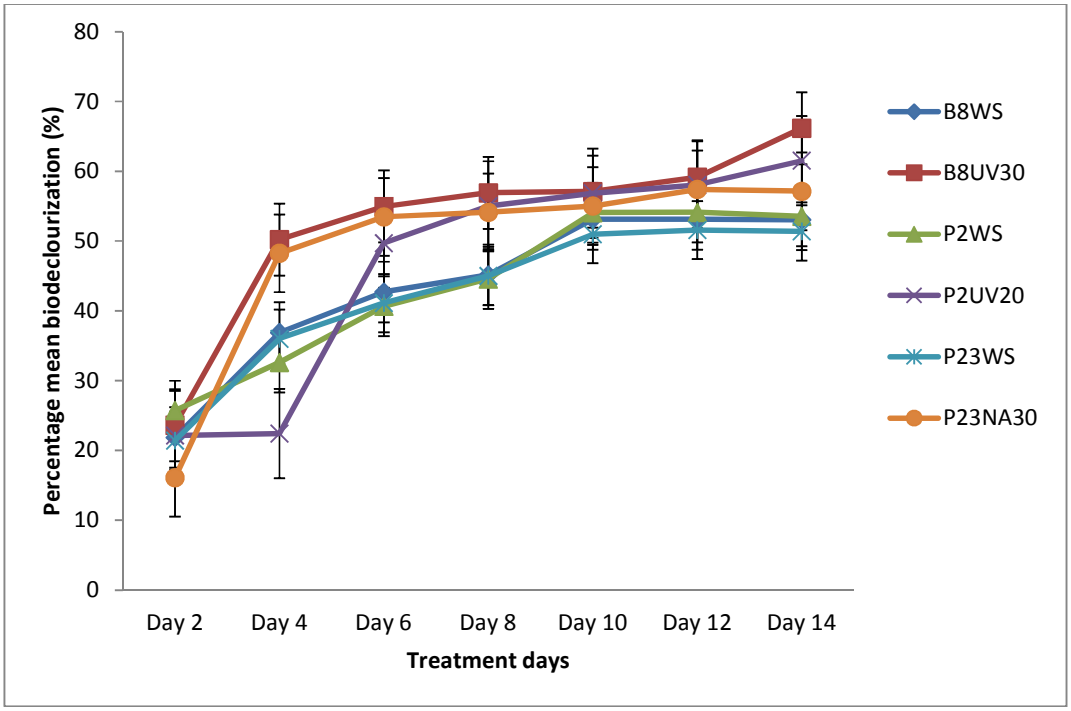

Fig. 7. The effects of incubation period on biodecolourization of textile effluent

Key: $B8_{UV}$ = UV-irradiated Bacillus cereus; $P2_{UV}$ = UV- irradiated Pseudomonas aeruginosa; $P23_{NA}$= Nitrous acid treated Pseudomonas fluorescens; WS = Wild strains; Time of treatments = (20 and 30 minutes)

Results revealed that wild strains of *Bacillus cereus, Pseudomonas aeruginosa*, *Bacillus firmus* and *Pseudomonas fluorescens* achieved 42.56±0.12, 40.43±0.62, 39.31±0.28 and 36.01±1.36% decolourization of textile effluents respectively. The results agreed with work done by Husseiny, [29]. These isolates probably had acquired natural adaptation to survive in the presence of the textile effluent. The ability of the organisms to degrade textile effluents is as a result of their metabolic activities.

Biodecolourization activities of the wild strains were enhanced by genetic improvement of the organisms using mutation technique. The technique has been widely employed to obtain strains with desired characteristics [30]. This was achieved by a combination of physical and chemical mutagens using ultraviolet irradiation and nitrous acids. The selections of $B8_{UV}30$, $P2_{UV}20$ and $P23_{NA}30$ for further studies were based on them having different amino acids requirement when compared with the parents. The treatment of organisms with mutagenic substances does not necessarily result in mutants being produced as the organisms possessed various mechanisms for reversal of such actions through repair of the damaged DNA. For example photo-reactivation could occur which consist of a single enzyme that can split pyrimidine dimmers in the presence of light, a process catalysed by photolyase enzyme which is found in many bacteria [31]. This is usually avoided by incubation of UV-irradiated organism for 5 hr with 2% caffeine in the dark [28]. Nitrous acid treatment, base excision repair (BER) could be employed in the removal of the damaged or inappropriate base from its sugar with glycosidase enzyme while a new base is incorporated by DNA polymerase using the other strand as a template [31,32].

Mutant strains $B8_{UV}30$ obtained after 30 minutes irradiation with a percentage survival of 3.00% gave the highest biodecolourization of 60.30±0.02%. This finding is in agreement with the observation of Ado et al. [28] that for a mutagenic agent to be successful, it should have a high rate of kill. The improved quality of textile effluents biodecolourization observed during this study after introduction of mutant strains, is attributed to mutation of parent strains. This corroborates the reports of previous studies by WHO, [33]; Ado et al. [28]; Idise et al. [22].

Temperature has a significant influence on biodecolourization of textile effluent by micro-organisms [19]. The effects of temperature revealed the maximum percentage bio-degradation of 53.92±0.79% at 35℃. This result supported the work done by Bisht et al. [34]. The mutagenised strains showed higher performance than the parent isolates at a higher temperature of 45℃. This could be as a result of mutation which made the mutant strains biodecolourization enzyme more resistant to temperature.

The effects of pH results revealed that the maximum percentage of decolourization was 58.82±1.02 at pH7. This result supported the concept of Pandey et al. [30] that better bacteria growths usually occur at pH7. However $B8_{UV}30$ at pH10 resulted slightly higher 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 [24].

The comparative study between the efficiency of static and shaking condition (150 rpm) for performance of the wild strains and parent strains on the decolourization of textile effluent revealed that the highest percentage biodecolourization (58.37±0.10%) by $P23_{NA}30$ was obtained under static condition. It was found that static conditions were more efficient than the shaking condition for both parent and mutant strains. This results are similar to those obtained by Daneshrar et al. [35] using another type of microorganism. High rate of agitation decrease bacterial growth and the activities of some biological substance such as enzymes which play important roles in the decolourization of the dye [36,37].

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. This was in agreement with the work conducted by Sarawasthi and Balakumar [21]. This implies that the organisms could not metabolize the dye as sole source of carbon and energy to appreciable level.

This work contradicted the previous work done by Murugalatha et al. [38] that organisms were able to show maximum level of textile effluent biodecolourization within 90 days of incubation as compared to highest obtained at 14 day. The effects of incubation period showed decolourization rate of 66.16±0.12% was

achieved at a period of 14 days. In this study, biodecolorization proceeded gradually up 14th days in the mutated strains whereas in the wild strains treatment, there was rapid decolourization during the initial days of exposure by the parent strains that slowed down abruptly on day 5th to 7th day of treatment. This is in agreement with work done by Mutambanengwe [39].

5. CONCLUSION

Environmental factors from this study proved to have an effect on the biodecolourization activities of the mutagenised strains of *Bacillus cereus*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* which resulted in increased decolourization efficiency of textile effluents as compared to the parent strains. They were able to biodecolourize appreciable level of textile effluent within a shorter period of 14 days. The mutant of organisms showed high tolerance to temperature and pH. The result obtained from this work shows, adequate manipulation of environmental condition could result to greater textile effluent decolourization by these organism. Hence, the introduction of these strains especially their enzymes in the wastewater treatment plant could be a plausible solution to the environment pollution problem therein in the discharge of the textile effluent currently.

6. RECOMMENDATION

Further investigations using other forms of mutagens should be conducted on other species of bacteria and fungi to build up a data bank of biodecolourization organisms. It should also be interesting to identify, isolate and concentrate the active enzymes of biodecolourization in the organism studied.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mendez-Paz D, Omil F, Lema JM. Anaerobic treatment of azo dye Acid Orange 7 under batch conditions. Enzyme Microbial Technology. 2004;36:264–272.
2. Stolz A. Basic and applied aspects in the microbial degradation of azo dyes. Applied Microbiology Biotechnology. 2001;56:69–80.
3. Pearce CI, Lloyd JT, Guthrie JT. The removal of colour from textile wastewater using whole bacteria cells: A review. Dyes and Pigments. 2003;58:179–196.
4. Moreira MT, Viacava C, Vidal G. Fed-batch decolourisation of poly R- 478 by *Trametes versicolor.* Brazilian Arch. Biology Technology. 2004;47(2):179–183.
5. John WC, Anyanwu NCJ, Akande D, Ayisa TT. Biodecolourization of textile effluent using mutagenised strains of *Pseudomonas* and *Bacillus* species isolated from dyed contaminated soil. IOSR Journal of Environmental Science, Toxicology and Food Technology. 2013;6(3):69-74.
6. Maier J, Kandelauer A, Erlacher A, Cavaco-Paaulo A, Gubitz GM. A new alkali thermostable azoreductase from *Bacillus* sp. Strain SF. Applied Environmental Microbiology. 2004;70(2):837-844.
7. Manu B, Chaudhari S. Anaerobic decolourisation of simulated textile wastewater containing azo dyes. Bioresearch Technology. 2002;82:225-231.
8. Bell J, Plumb JJ, Buckley CA, Stuckey DC. Treatment and decolourisation of dyes in an anaerobic baffled reactor. Journal of Environmental Engineering. 2000;126(11): 1026–1032.
9. Seshadri S, Bishop PL, Agha AM. Anaerobic/aerobic treatment of selected azo dyes in waste water. Waste Management. 1994;14(2):127–137.
10. Banat IM, Nigam P, Singh D, Marchant R. Microbial decolourisation of textile dye containing effluents: A review. Bioresearch Technology. 1996;58:217–227.
11. Kumar K, Devi SS, Krishnamurthi K, Gampawar S, Mishra N, Pandya GH, Chakrabarti T. Decolorisation, biodegra-dation and detoxification of benzidine based azo dye. Bioresour Technol. 2006;97:407–413.
12. Sponza DT, Isik M. Decolourisation and azo dye degradation by anaerobic/aerobic sequential process. Enzyme Microbial Technology. 2002;31:102–110.
13. Pagga U, Brown D. The degradation of dyestuffs part II: behaviour of dyestuffs in aerobic biodegradation tests. Chemosphere. 1986;15(4):479–491.

14. Porter JJ. Recovery of polyvinyl alcohol and hot water from the textile wastewater using thermally stable membranes. J. Membr. Sci. 1998;151:45-53.
15. Sapci Z, Ustun B. The removal of color and cod from textile wastewater by using waste pumice. EJEAF Chemistry. 2003;2:286-290.
16. Melgoza RM, Cruz A, Buitron G. Anaerobic/aerobic treatment of colorants present in textile effluents. Water Science Technology. 2004;50:149-155.
17. Andleeb S, Atiq N, Ali MI, Hussnain RR, Shafique M, Ahmad B, Ghumro PB, Hussain M, Hameed A, Ahmad S. Biological treatment of textile effluent in stirred tank bioreactor. International Journal of Agricultural Biology. 2010;12: 256-260.
18. McMullan G, Meehan C, Coneely A, Kirby N, Robinson T, Nigan P, Banat IM, Marchant R, Symth WF. Microbial decolorization and degradation of textile dyes. Applied Microbiology Technology. 2001;56:81-87.
19. Samuel EA, Ayobami OA. Evaluation of microbial system for biotreatment of textile waste effluent in Nigeria. Biodecolourization and biodegradation of textile dye. Journal of Aplied Science Environmental Management. 2011;15(1): 79-86.
20. Roy R, Fakhruddin ANM, Khatun R, Islam MS, Ahsan AM, Neger AJMT. Characterization of textile industrial effluents and its effects on aquatic macrophytes and algae. Bangladesh J. Sci. Ind. Res. 2010;45:79–84.
21. Saraswathi K, Balakumar B. Biodecolourization of azo dye (pigmented red 208) using *Bacillus firmus* and *Bacillus laterosporus*. Journal of Bioscience Technology. 2009;1:1-7.
22. Idise OE, Ameh JB, Yakubu SE, Okuofo CA. Modification of *Bacillus cereus* and *Pseudomonas aeruginosa* isolated from a petroleum refining effluent for increased petroleum product degradation. African Journal of Biotechnology. 2010;9(22).
23. Iftikhar T, Niaz M, Abbas SQ, Zia MA, Ashraf I, Lee KJ, al Haq I. Mutation induced enhanced biosynthesis of lipases by *Rhizopus Oligosporus Var. Microsporus*. Pak. Journal bot. 2010; 42(2):1235-1249.
24. Kalyanee J, Rujikan N, Jongjira N, Boonsiri C. Decolourization and degradation of C I reactive Red 195 by *Enterobacter species*. Thammasat International Journal of Science Technology. 2008;12(4).
25. Ezeronye OU, Asamudo NU, Daba AS. Bioremediation of textile effluent using *Phanerochaete chrysosporium*. African Journal of Biotechnology. 2005;4(13): 1548-1553.
26. Olukanni OO, Osuntoki AA, Gberile GO. Textile effluent biodegradation potentials of textiles effluent-adapted and non-adapted bacteria. African Journal of Biotechnology. 2006;5(20):1980-1984.
27. Chen KJ, Wua D, Liou S, Hwang J. Decolourization of the textile dyes by newly isolated bacterial strains. Journal of Biotechnology. 2003;101:57-68.
28. Ado SA. Studies on genetic improvement of *Aspergillus niger* – CAS for amylase production. PhD thesis, Department of Microbiology, Ahmadu Bello University, Zaria; 2004.
29. Husseiny SM. Biodegradation of the reactive and direct dyes using Egyptian isolates. Journal of Applied Sciences Research. 2008;4(6):599-606.
30. Pandey A, Singh P, Iyengar L. Bacterial decolourization and degradation of azo dyes. Int. Biodeterior. Biodegrad. 2007;59(2):73–84.
31. Anon. Important water quality factors; 2009a.
Available:http//www.Important water quality factors
32. VanHamme JD, Singh A, Ward OP. Recent advances in Petroleum micro-biology. Molecular Biology. Reviewed. 2003;67(4):503-549.
33. WHO Water pollutants, biological agents, dissolved chemicals non dissolved chemicals, sediments, heat, WHO ceha, amman, Jordan; 2002.
34. Bisht S, Pandey P, Sood A, Sharma S, Bisht NS. Biodegradation of naphthalene and anthracene by chemo-tactically active rhizobacteria of populus deltoids. Brazilian Journal of Microbiology. 2010;41:922-930.
35. Daneshvar N, Ayazloo M, Khatae AR, Pourhassan M. Biological decolourization of dye solution containing Malachite green by microalgae *Cosmarium* sp. Bioresource Technology. 2007;98:1-7.
36. Faison BD, Kirk TK. Factors involved in the regulation of a ligninase activity in *Phanerochaete chrysosporium*. Appleid Environment Microbiology. 1985;30:953-304.

37. Ge Y, Yan L, Qing K. Effect of environment factors on dye decolourization by Penicillium sordid AATCC 90872 in an aerated reactor. Process Biochemistry. 2004;7:1401-1405.
38. Murugalatha N, Mohankumar A, Rajestic Sankaravadiro A. Textile effluent treatment by bacillus species isolated from processed food. African Journal of Microbiology Research. 2010;20:2122-2126.
39. Mutambanengwe CCZ, Togo CA, Whitely CG. Decolorization and degradation of textile dyes with biosulfidogenic hydrogenases. Biotechnology Programme. 2007;23:1095-1100.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14552