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Studies on Physicochemical Analysis and Biodecolorization Potential of Some Bacteria Isolated from Textile Effluent

A. Bello[1*], M. D. Makut[1] and G. O. Ogah[1]

[1]Department of Microbiology, Nasarawa State University, Keffi, Nasarawa State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MDM and GOO managed the analyses of the study. Author GOO managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aim: This research work is aimed at studies on physicochemical analysis and biodecolorization potential of some bacteria isolated from textile effluent.
Study Design: This study is designed to isolate and identify species of bacteria from textile effluent. To determine the physicochemical properties of the textile effluent. To use bacteria isolates in biodecolorization of the textile effluent. To determine the physicochemical properties of the textile effluent after treatment with bacterial isolates using methods described by the American public health association. To biodecolorize textile effluents at three different concentrations.
Place and Duration of Study: Department of Microbiology, Nasarawa State University Keffi, Nasarawa State, Nigeria, between September 2016 and October 2017.
Methodology: Samples of textile effluent were collected in clean containers, the physicochemical properties were analyzed using standard methods described by the American Public Health Association and compared with those of national and international standards. These textile effluents were subjected to bacterial decolorization using a decolorization medium, composed of

**Corresponding author: E-mail: ameenubello91@gmail.com;*

minimal salt medium and textile effluent for a period of 15 days, the %decolorization was measured by checking absorbance of the sample at 72 hours intervals using a UV- spectrophotometer. This was repeated at different concentrations of 20 ml/250 ml, 20 ml/500 ml, and 20 ml/1000 ml (v/v of textile effluent and minimal salt medium).

Results: The bacteria isolates used were *Bacillus subtilis, Pseudomonas aeruginosa*, *Micrococcus luteus*. The physicochemical properties of the effluent changed after 15 days of bacterial activity, at best; the pH was reduced from 9.8 to 7.2, color from black to clear/colorless, turbidity from 9,750 to 1,201NTU, TDS from 22,800 to 3,351 mg/L, conductivity from 33,800 to 5,001 µs/cm, sulphate from 975 to 91 mg/L, phosphates from 2.75 to 0.86 mg/L, COD from 3,550 to 195 mg/L, BOD from 1,425 to 78 mg/L. The highest %decolorization was observed to be 53.56% by *B. subtilis*, followed by 50.95% by *P. aeruginosa*, and 47.97% by *M. luteus*.

Conclusion: From this study, it can be deduced that the use of bacteria in biodecolorization has shown great potential and also improves the physicochemical properties of the effluent. However, there is need for further work to be done to validate and improve these findings.

Keywords: Biodecolorization; decolorization; textile effluent; Bacillus subtilis; Pseudomonas aeruginosa; Micrococcus luteus.

1. INTRODUCTION

Many industries usually need water in large volumes. However, only a small amount of this water is consumed. A large portion of it is usually discarded as effluent. The textile factories as a case study generates large amount of textile effluent during its production processes which include grinning, spinning, weaving etc. This processes which finally lead to generation of large volumes of wastewater [1]. Textile effluent when disposed into the environment poses serious environmental problems most especially in developing countries where portable water is hard to come by. Color removal particularly, has recently become a major scientific interest, as indicated by the multitude of related research reports. Textile industries produce effluents containing a number of chemicals (dispersants, leveling agents, acids, alkalis and various dyes) [2]. In Nigeria, textile factories indiscriminately discharge large amounts of effluent into public drains which eventually find its way into larger water bodies like rivers. All as a result of strictures and governing laws. Although several methods have been described in treatment of textile effluent to achieve color removal, a lot of these factories are yet to imbibe these practices [3]. Physiochemical methods such as filtration, specific coagulation, use of activated carbon and chemical flocculation have been used in treatment of these textile effluents. Some of these methods include physicochemical methods which are effective in direct decolorization but can be expensive and have problems such as low efficiency and in applicability to a wide range of dyes. However, biological methods of treatment are a cheaper alternative which is also more environments friendly [4,5].

2. MATERIALS AND METHODS

2.1 Sample and Sample Collection

Textile effluent samples were collected in 10 liters sterile containers from Funtua textile mill, Katsina State placed in an ice box and transported to the laboratory without any pre-treatment or chemical addition, while maintaining the highest aseptic techniques and stored at 4°C according to Joshi et al. [6]. Some physicochemical parameters of the textile effluent *viz.,* temperature, pH, color and smell were measured.

2.2 Physicochemical Analysis of Textile Effluent

The physicochemical parameters of the textile effluent were analyzed respectively using methods described by the American Public Health Association (APHA), 2012 [7] and the results were compared to standards of World Health Organization (WHO), Environmental Protection Agency (EPA), and Nigerian Standard for Drinking Water Quality (NSDWQ). These parameters include; pH using JENWAY model 3020 pH meter, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD) to determine the amount of biologically active pollutants in the textile effluent, temperature, total dissolved solids, phosphates, nitrates, sulphate, conductivity, and turbidity.

2.3 Microbiological Analysis of the Textile Effluent

The microbiological analysis of the textile effluent was carried out by serially diluting the textile effluent. The diluents 10^4, $I0^5$, and 10^6, were inoculated on nutrient agar plates for the isolation of bacteria and incubated at 37°C for 24 hours. Amphotericin B was added to the culture plates to inhibit growth of fungal andyeast cells. The bacterial growth was counted in colony forming units.

2.4 Enumeration of Bacteria

Bacteria were enumerated by serial dilution. One milliliter (1 mL) of textile effluent was aseptically introduced into 9 ml of distilled water in a test tube, shaken and serially diluted. One milliliter (1 mL) each was inoculated on sterile petri dishes and sterile Nutrient Agar was added to the petri dishes using the pour plate technique as described by [8] and thoroughly mixed for the enumeration of bacteria. The plates were incubated for 24h at 37°C after which the colonies were counted and recorded as colony forming units per milliliter (cfu/mL) of effluent. The isolates were sub cultured repeatedly on nutrient agar. The pure isolates were preserved on Nutrient agar slants for further characterization and identification. Characterization and identification of the bacteria isolates was done by physical macroscopic examination of their cultural characteristics such as texture, color and other morphological characteristics. Gram staining technique was also used to identify the bacterial Gram's reaction. Biochemical tests such as: starch hydrolysis, catalase, citrate utilization, motility, VogesProskauer, glucose, lactose, maltose and mannitol fermentation, indole and methyl red were carried out for confirmation of the exact bacteria. The identity of each isolate was confirmed by comparing their characteristics with those of known taxa using Bergey'sManualsof Determinative Bacteriology. Pure isolates were then transferred onto fresh medium for proper storage [9].

2.5 Preparation of Decolorization Medium

After preparation of minimal salt medium, textile effluent (20 mL) was added to 250 mL, 500 mL and 1000 mL of the Minimal salt medium a modification of the methods described by Srinivasan et al. [10] pH of the medium adjusted to neutral using a pH meter with 0.1M HCl and 0.1M NaOH as buffer solutions. The medium was sterilized by autoclaving at 121°C for 15 minutes. It was then allowed to cool until ambient temperature.

2.6 Textile Effluent Decolorization

The decolorization experiment was carried out in 50 mL Erlenmeyer flask. 20 mL of the decolorization media were dispensed into 50 mL Erlenmeyer flasks and 2 mL of 24 hr bacterial isolates were inoculated into their pre-labeled respective flasks. A control was also prepared to which no isolates were added before being incubated at 37°C for a period of 360 hrs (15 days). Experiments were done in triplicates, maintaining the highest aseptic conditions [10]. Samples were drawn at 72 hrs interval for observation. 3 mL of the mixture was centrifuged at 5000 rpm for 15 minutes. The decolorization of the effluent was determined by measuring absorbance of the supernatant with the use of a UV- spectrophotometer at a wavelength of 720 nm. The percentage decolorization was calculated using the equation below:

$$\%\text{Decolorization} = \frac{\text{initial decolorization} - \text{final decolorization}}{\text{initial decolorization}} \times 100$$

2.7 Determination of the Effect of Concentration on Decolorization

Different concentration gradients of 250 ml, 500 ml and 1000 ml were created by dispensing equal amounts of textile effluent (20 ml) into these amounts of MSM. Two milliliters of 24 hr bacterial isolates were inoculated into their respective pre-labeled flasks. A control was also prepared for each concentration to which no isolates were added. All works were done in triplicates, maintaining the highest aseptic conditions before being incubated at 37°C for a period of 15 days [10]. Samples were drawn at 72 hrs interval for observation. 3 ml of the mixture was centrifuged at 5000 rpm for 15 minutes. The decolorization of the effluent was determined by measuring absorbance of the supernatant with the use of a UV-spectrophotometer at a wavelength of 720 nm. The percentage decolorization was calculated using the equation stated above.

3. RESULTS AND DISCUSSION

3.1 Microbial Count of the Isolates

The microbial count of the isolates in the textile effluent samples used for the analysis was

carried out. The 10^4 dilution factor was plated on Nutrient Agar in triplicates and incubated at 35°C for 24 hours. The number of colony forming units per plate was counted and the average was calculated.

Table 1. Number of colony forming units on different plates at 10^4 dilution factor

Sample	Colony forming units (CFU/mL)
Effluent 1	$4.2X10^5 \pm 3$
Effluent 2	$3.6X10^5 \pm 4$
Effluent 3	$2.9X10^5 \pm 4$

3.2 Bacterial Isolates

Bacillus subtilis, Pseudomonas aeruginosa, and Micrococcus luteus were isolated from the textile effluent. These isolates where characterized based on their appearance, morphological and biochemical characteristics.

Fig. 1 shows the percentage decolorization of treated textile effluent at 20 ml/250 ml by *Bacillus subtilis*, *Micrococcus luteus* and *Pseudomonas aeruginosa* respectively. The overall highest percentage decolorization (53.56%) was observed at day 15 by *B. subtilis* followed by *P. aeruginosa* (50.95%) at day 15 and *M. luteus* (47.97%) also at day 15. From the results, it is obvious that as the incubation period increased the percentage decolorization also increased.

Fig. 2 shows the percentage decolorization of textile effluent at 20 ml/500 ml. The overall highest was observed at day 15 by *M. luteus* (23.03%) followed by *B. subtilis* (22.96%) and *P. aeruginosa* (20.48%).

Table 2. Physicochemical parameters of raw textile effluent physicochemical parameters of raw textile effluent in comparison with standard limits for EPA, WHO and NSDWQ

Parameters	Unit	Raw effluent	EPA	WHO	NSDWQ
Temperature	°C	40	40	-	-
pH	-	9.8	7.0	7.0	7.0
Color	-	Black	Clear	Clear	Clear
Odor	-	Rotten Egg	Odorless	Odorless	Odorless
Turbidity	NTU	9,750	<10	5	5
TDS	mg/L	22,800	2000	2000	500
Conductivity	µS/cm	33,800	70	5	1000
Sulphate	mg/L	975	750	400	100
Phosphate	mg/L	2.75	-	-	3.5
COD	mg/L	3,550	120	200-1000	30
BOD	mg/L	1,425	40	20	50

Key: EPA- Environmental protection agency, WHO World Health Organization, NSDWQ- Nigerian Standard for Drinking Water Quality, BOD- Biochemical oxygen demand, COD- Chemical oxygen demand, TDS-Total dissolved solids

Table 3. Physicochemical parameters of textile effluent treated with bacteria isolates

Parameters	Unit	*B. subtilis*	*M. luteus*	*P. aeruginosa*
Temperature	°C	34	33	36
pH	-	7.8	7.2	8.2
Color	-	Clear	Clear	Light green
Odor	-	-	-	-
Turbidity	NTU	1,201	1,789	1,822
TDS	mg/L	3,351	4,053	4,117
Conductivity	µS/cm	5,001	6,050	7,002
Sulphate	mg/L	91	100	93
Phosphate	mg/L	0.86	0.97	0.74
COD	mg/L	195	232	1,443
BOD	mg/L	78	93	280

BOD- Biochemical oxygen demand, COD- Chemical oxygen demand, TDS-Total dissolved solids

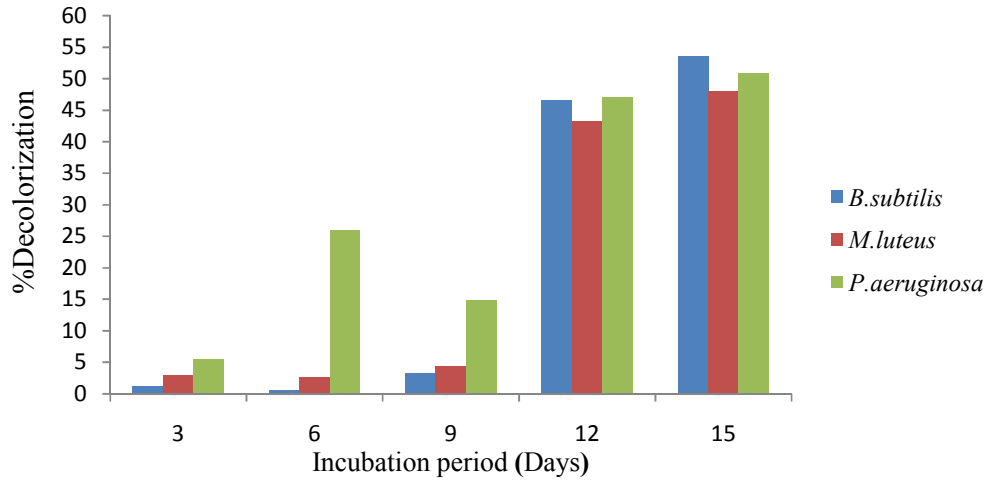

Fig. 1. Percentage decolorization of textile effluent at 20 ml/250 mL

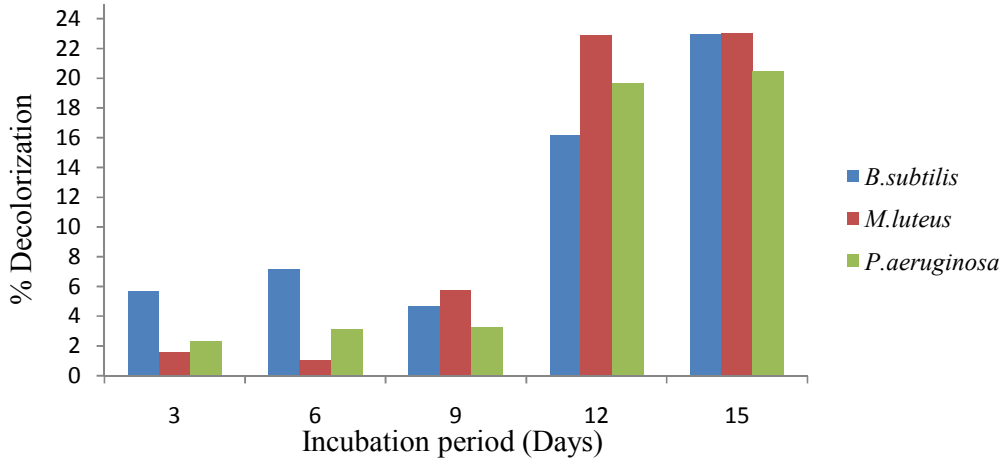

Fig. 2. Percentage decolorization of textile effluent at 20 ml/500 mL

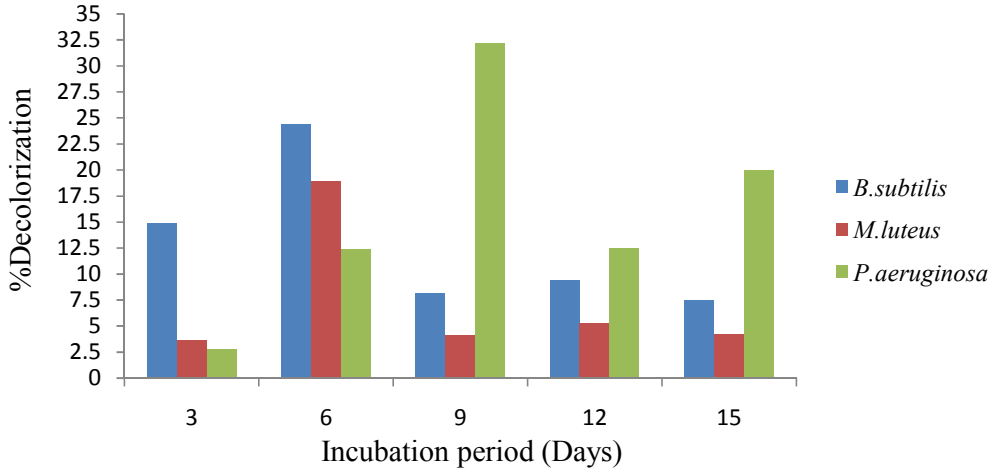

Fig. 3. Percentage decolorization of textile effluent at 20 ml/1000 mL

Fig. 3 shows the decolorization of textile effluent at 20 ml/1000 ml. The highest percentage decolorization was observed at day 9 by *P. aeruginosa* (32.18%) followed by *B. subtilis* (24.36%) on day 6, and *M. luteus* (18.89%) on day 6.

4. CONCLUSION

With constant innovations in the textile industries, air and water pollution are continually on an incline as a result of the synthetic dyes and textile production processes. Unless restrictions are set and positive steps are taken, this is bound to continue. The negative effect of large amounts of chemicals and dyestuff in the environment is indeed serious, because their effects are usually not instantaneous and the best way to tackle this is biologically, which is less likely to lead to formation of more toxic compounds. There is need for an immediate and effective solution to the problem of environmental pollution resulting from the activities of textile factories. If we are to protect the populace from chemical exposure and health problems resulting from chemical exposure, we all need to go green and do what is best for our environmental health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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