

Short Research Article

**STUDIES ON BIODECOLORIZATION OF
TEXTILE EFFLUENT USING *BACILLUS
SUBTILIS* AND *PSEUDOMONAS AERUGINOSA*
ISOLATED FROM TEXTILE EFFLUENT**

ABSTRACT

Aim: This research work is aimed at Studies on Biodecolorization of Textile Effluent Using *Bacillus Subtilis* and *Pseudomonas Aeruginosa* Isolated From Textile Effluent.

Study design: This study is designed to isolate and identify *Bacillus Subtilis* and *Pseudomonas aeruginosa* from textile effluent. To use bacterial isolates in biodecolorization of the textile effluent individually and in consortium (both isolates together) at three different concentration.

Place and duration of study: Department of Microbiology, Nasarawa State University Keffi, Nasarawa State, Nigeria, between August 2016 and November 2017.

Methodology: Textile effluent was collected in a screw capped sterilized bottle from the textile mill discharge point. The bacterial of choice were isolated from the textile effluent and were subjected to bacterial decolorization individually and then in consortium using a decolorization medium, composed of minimal salt medium and textile effluent for a period of 12 days, the %Decolorization was measured by checking absorbance of the sample at 72hours intervals using a UV- spectrophotometer (720nm wavelength). This was repeated at different concentrations of 20ml/250ml, 20ml/500ml, and 20ml/750ml (v/v of textile effluent and minimal salt medium).

Results: *Bacillus subtilis* and *Pseudomonas aeruginosa* were found to have significant potential to decolorize the dye effluent. *Bacillus subtilis* produced high decolorization activity after 12 days with 58.78% at 20ml/750ml concentration. Whereas, *Pseudomonas aeruginosa* produced 53.46% at 20ml/750ml concentration after 12 days period of incubation. *Pseudomonas aeruginosa* and *Bacillus subtilis* in consortium produced the highest decolorization potential of 75.56% at concentration 20ml/750ml after 12 days period of incubation.

Conclusion: This study shows that *Bacillus subtilis* has comparatively higher decolorization potential than *Pseudomonas aeruginosa*. The bacteria in consortium exhibited the highest ability in decolorizing process than the individual isolates. However, there is need for further work to be done to validate and improve these findings.

Keywords: Biodecolorization, Textile effluent, *Bacillus subtilis*, *Pseudomonas aeruginosa*, consortium .

1.0 INTRODUCTION

Steady growth of industries has precipitated the use of diverse chemicals in our daily lives. Textile industry form part of the industries that uses synthetic chemicals such as Azo dyes [1]. Azo dyes which are aromatic compounds are increasingly used in industries due to their simple and less expensive. About 5-10% of the azo dyes are discharged in environment as dyestuff waste [2]. Different methods of waste water treatment techniques such as filtration, coagulation, activated carbon and chemical flocculation have been put forward for the removal of colored textile effluent [3]. All these methods are very costly and can result in the production of large quantity of waste water, these lead to secondary level pollution of the land .However, these treatments are not found effective against the removal of color and chemicals used in the industry [4]. Safe removal of the color dye polluting the environment is necessary. Cleaning up the environment through microorganisms (Bioremediation) has been known as less expensive and ecofriendly method for disposal of textile effluent [5]. Since Textile industries produce large amounts of liquid wastes that contain organic and inorganic compounds. During the dyeing processes, not all dyes that are applied to the fabrics are fixed on them and there is always a portion of these dyes that remains unfixed to the fabrics and gets washed out [6].

2.0 MATERIALS AND METHODS

2.1 Sample and Sample Collection

Textile effluent was collected from Teritex (No. 2 Bompai road Kano, Kano State). The textile effluent was collected in a screw capped sterilized bottle from the textile mill discharge point and was brought to the laboratory with ice pack in a cooler box and stored at 4°C [7].

2.2 Isolation of *Bacillus subtilis* and *Pseudomonas aeruginosa*

Bacillus subtilis and *Pseudomonas aeruginosa* were isolated from the textile effluent. Briefly, 1.0ml of the textile effluent was serially diluted up to 7-folds (10^{-7}) in 9ml of distilled water and pour plate technique was used to culture the bacteria on Nutrient Agar at 37°C for 24 hours [7].

2.3 Identification of *Bacillus subtilis* and *Pseudomonas aeruginosa*

After incubation, the bacterial colonies were observed. All colonies were isolated, sub- cultured and identified using several morphological and biochemical tests including Gram's staining, motility test, voges-proskauer , methyl red test, citrate utilization test, starch hydrolysis test, oxidase test, and catalase test using the taxonomic scheme of Berger's manual of determinative Bacteriology.

2.4 Sterilization of the Textile effluent

The textile effluent was sterilized after the bacteria isolates were isolated from the effluent in 1000ml Erlenmeyer flask using the autoclave at 121°C for 15 minutes. This is done to prevent interference of other microorganism that might be present in the effluent from interacting with the choice isolates.

2.5 Media preparation

Minimal salt medium (MSM) prepared Contained the following: $(\text{NH}_4)_2\text{SO}_4$ (500mg/l), CaCl_2 (14mg/l), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (120mg/l) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.13mg/l), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (5.0mg/l), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (2.5mg/l), $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (700mg/l), KH_2PO_4 (400mg/l), pH (7), NaCl (1mg/l) and Glucose (10mg/l) [8].

2.6 Decolorization Medium

After preparation of minimal salt medium, it was sterilized by autoclaving at 121°C for 15 minutes. It was then allowed to cool until ambient temperature is achieved before inoculating with test organism. 20ml of the collected textile effluent was added to a separate 250ml, 500ml, and 750ml minimal salt medium .

2.7 Decolorization by individual isolates

Decolorization experiment was carried out in 50ml Erlenmeyer flask. Different concentration gradients of 250ml, 500ml and 750ml of the minimal salt medium were created by dispensing equal amounts (20ml) of textile effluent into these amounts of MSM.. 20ml were then dispensed into 50ml Erlenmeyer flasks and 2ml of 24hr bacterial isolates were inoculated into their respective 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 12 days [9].

2.8 Decolorization by consortium of bacterial isolates

The decolorization experiment was carried out in 50ml Erlenmeyer flask. Different concentration gradients of 250ml, 500ml and 750ml of the minimal salt media were created and equal amounts of textile effluent (20ml) was added to the MSM. 20ml were then dispensed into 50ml Erlenmeyer flasks. 2ml (1ml each) consortiums of 24hr bacterial isolates were inoculated into the decolorization medium. 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 12 days [9].

2.9 Decolorization Assay

During incubation, Samples were drawn at 72hrs interval for observation. 4ml of the mixture was centrifuged at 5000rpm for 15minutes. The decolorization potential of the isolates was determined by taking the absorbance reading of the cell free supernatant using spectrophotometer at a wavelength of 720nm.

The extent of decolorization was expressed as percentage (%) decolorization and estimated as;

$$\% \text{ Decolorization} = \frac{A_o - A_t}{A_o} \times 100\%$$

Where A_o is the initial absorbance of the textile effluent in ml/l

A_t is the final absorbance of the textile effluent in ml/l respectively [10].

3.0 Results and Discussion

3.1 Bacterial isolates

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

Figure 1 shows the percentage decolorization of treated textile effluent at 20ml/250ml by *Bacillus subtilis*, *Pseudomonas aeruginosa* and bacteria in consortium respectively. The overall highest percentage decolorization (63.19 %) was observed at day 12 by bacteria in consortium followed by *Bacillus subtilis* (47.04%) at day 12 and *Pseudomonas aeruginosa* (46.54%) also at day 12. From the results it shows that as the incubation period increased the percentage decolorization increased and decrease in the concentration of the textile effluent increases the decolorization ability.

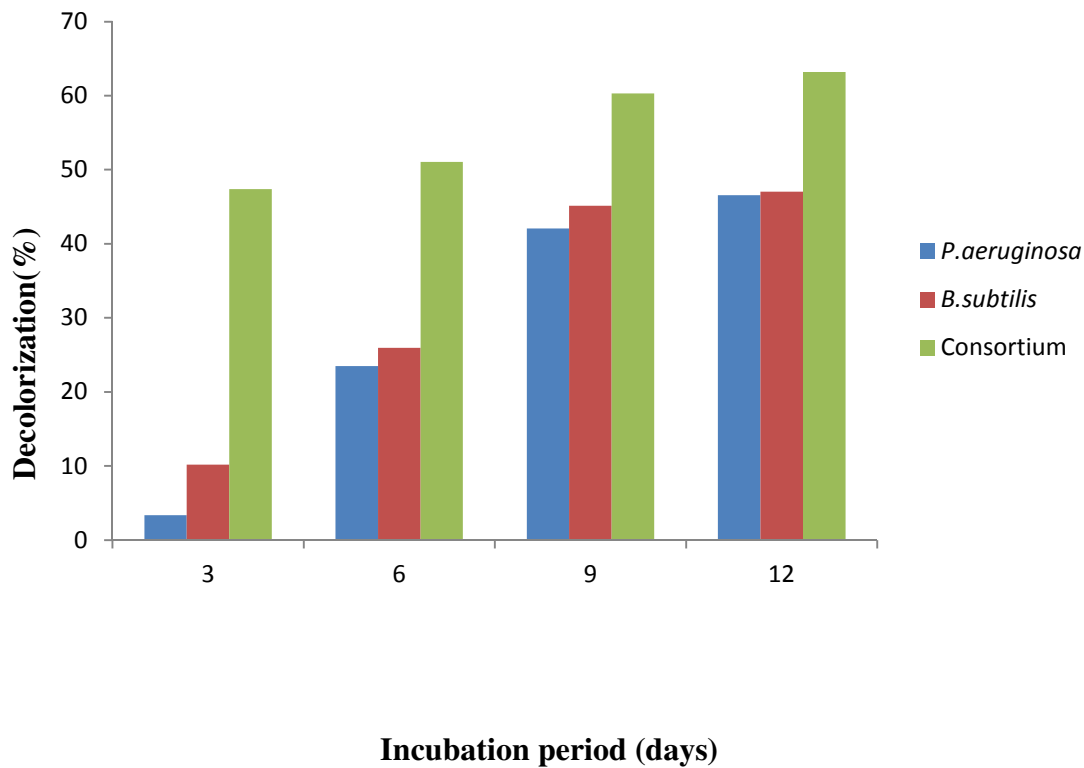
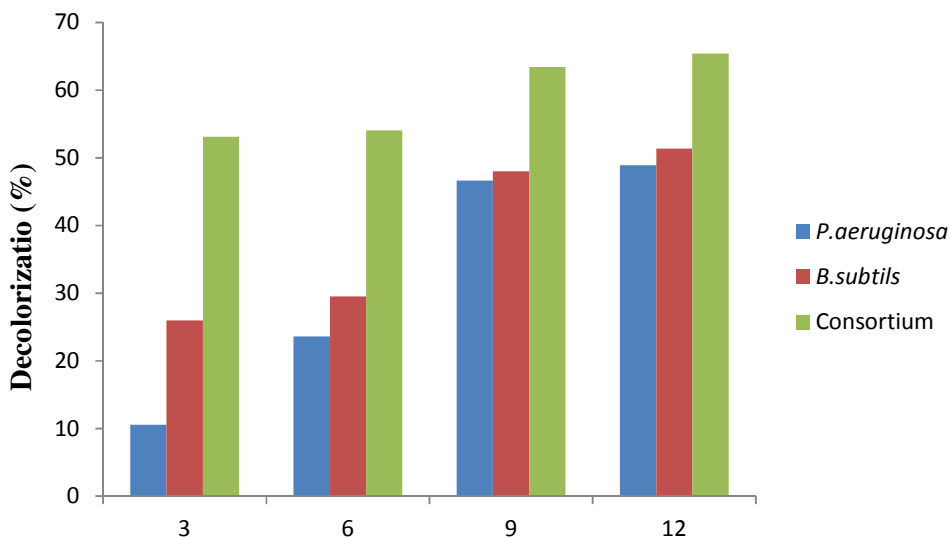


Figure 1. Percentage Decolorization of textile effluent at 20ml/250ml

Figure 2 shows the percentage decolorization of treated textile effluent at 20ml/500ml by *Bacillus subtilis*, *Pseudomonas aeruginosa* and bacteria in consortium respectively. The overall highest percentage decolorization (64.41 %) was observed at day 12 by bacteria in consortium followed by *Bacillus subtilis* (51.35%) at day 12 and *Pseudomonas aeruginosa* (48.88%) also at day 12.



Incubation period (days)

Figure 2. Percentage Decolorization of Textile effluent at 20ml/500ml

Figure 3 shows the percentage decolorization of treated textile effluent at 20ml/750ml by *Bacillus subtilis*, *Pseudomonas aeruginosa* and bacteria in consortium respectively. The overall highest percentage decolorization (75.56 %) was observed at day 12 by bacteria in consortium followed by *Bacillus subtilis* (58.78%) at day 12 and *Pseudomonas aeruginosa* (53.46%) also at day 12.

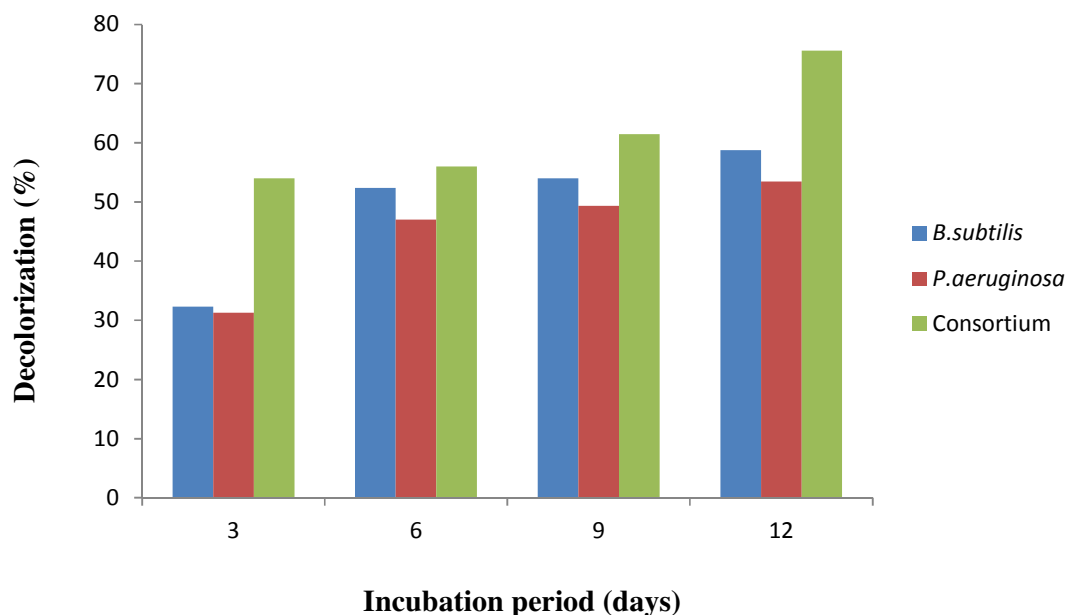


Figure 3. Percentage Decolorization of Textile effluent at 20ml/750ml

4.0 Conclusion

Application of traditional waste water treatment requires enormous cost and continuous input of chemical which becomes uneconomical and causes further environmental damage. Hence economical and eco-friendly techniques using bacteria can be applied for the tuning of waste water [11,12]. Biotreatment offers easy, cheaper and effective alternative for color removal of textile dyes. Thus, by this present study we strongly concluded that the bacterial isolates like *Bacillus subtilis* and *Pseudomonas aeruginosa* were as a good microbial source for waste water treatment specifically in biological decolorization of textile dye effluent.

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