

## BIOREMEDIATION OF EFFLUENT FROM LOCAL TEXTILE INDUSTRY USING *Bacillus licheniformis*

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**Abstract:** Microbial treatment of effluent from local textile industries was studied in this work. Effluent samples were collected from dye-houses at Itoku, Abeokuta (Southwestern, Nigeria). Bacteria were isolated from samples collected. The bacteria isolated were screened for their ability to decolourize dyes used in the local textile industries. The isolate with the highest decolourization ability on the dyes was used to decolourize effluents from the dye-houses. Gas Chromatography Mass Spectroscopy (GC-MS) analysis and Brine Shrimps Cytotoxicity test was carried out using cell free supernatant of the effluent to determine the degradative ability and the detoxification potential of the isolate. *Bacillus licheniformis* showed the best decolourization ability for the dyes having 90.32% and 63.76% decolourization potential on Gambia gold and army green respectively. It was therefore used in the treatment of effluent from local textile industries and it had 43.23% decolourization potential. GC-MS analysis of the *B. licheniformis* treated effluent revealed reduction in the percentage concentration of compounds such as quinoline and 7-methylquinoline which had a concentration of 19.74% and 5% in the untreated effluent and a final concentration of 2.85% and 0% respectively in the treated effluent. Also, compounds such as eicosane and eicosanoic acid which were absent in the untreated effluent were formed during the microbial treatment of the effluent as revealed by GC-MS analysis. Brine shrimps cytotoxicity test revealed a decrease in the cytotoxicity of the treated effluent which had an LC<sub>50</sub> of 642.72 compared to the untreated effluent which had an LC<sub>50</sub> of 1.61.

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### 1. Introduction

Textile industries are industries which carry out processes such as pretreatment of fibres or cloth materials, dyeing, printing and finishing operations. These production processes do not only consume large amounts of energy and water but they also produce a substantial amount of waste products (Babu *et al.*, 2007).

With the increased demand for textile products, the textile industry and its waste waters have increased proportionally making it one of the main sources of severe pollution problems worldwide (Andre *et al.*, 2007). The effluent generated does not only affect the people alone, but also the environment in terms of contamination of ground water which affects the natural hydrologic cycle. The textile industries produces effluents that contain several types of chemicals such as dispersants, leveling agents, acids, alkalis, carriers and various dyes. This alters the pH, increases the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and gives the rivers intense colourations. The use of these water resources is limited and the ecosystem is also affected.

The local textile industries in Nigeria where the effluents were collected do not operate any treatment method at all. Even when efforts are made to treat the effluents, physico-chemical treatment methods are used and these treatment methods are the least desirable owing to their high costs and generation of secondary pollutants. On the other

hand, biological treatment methods are attractive due to their cost effectiveness, diverse metabolic pathways and versatility of microorganisms used for treatment (Méndez-Paz *et al.*, 2005 and Pandey *et al.*, 2007). Some bacterial strains such as *Bacillus cereus*, *Pseudomonas putida*, *Pseudomonas fluorescence*, *Steanotrophomonas acidaminiphila* have been used in the biodegradation of azo dyes (Khehra *et al.*, 2006). Many of these strains require organic carbon sources as they cannot utilize dye as the growth substrate. There are some reports on complete degradation of sulfonated aromatic amines by enriched bacterial communities (Tan *et al.*, 2000).

This study therefore evaluated the ability of microbial isolate to decolourize, detoxify and degrade effluent generated from local textile industry.

### 2. Materials and Methods

#### Sample Collection:

**Dyes and effluents:** the dyes and effluents used in this study were obtained from Itoku in Abeokuta, Ogun State in the southwestern region of Nigeria. Dyes such as army green (mixture of green and black dyes) and Gambia gold (a mixture of brown and red dyes) obtained from this market were manufactured by Consignee-PJS Products Limited, Changzhou, Jiangsu, China.

**Brine Shrimps:** Cysts of *Artemia salina* from Great Salt Lake, Utah, USA were bought from a vendor selling aquaculture materials in Lagos.

Isolation, identification and screening of microbial isolates:

Microorganisms were isolated from effluent from local textile industries and soils contaminated with dye effluent. The isolates were characterized using morphological and biochemical tests described by Olutiola *et al.* (2000). Identification of the bacteria was carried out using Cowan and Steel (1993) and Holt *et al.* (2000). Screening for isolates with decolourization ability was done using modified method of Sharma *et al.* (2004) in which dyes used in the local textile industries such as army green and Gambia gold was added to the minimal salt media used.

Decolourization Study:

Minimal salt medium (MSM) supplemented with 100mg/L of the dye or effluent (Sharma *et al.*, 2004) were put into 150mL of Erlenmeyer flask and this was inoculated with 5mL of 24 hours old broth culture of the bacterial isolates and incubated for 7 days, after which the medium was centrifuged at 7,000 rpm at 4°C for 15 minutes using cold centrifuge, Mikro 220R, Hettich Zentrifugen model. The decolourization potential of the isolate was determined by taking the absorbance reading of the cell free supernatant using UV/Visible Spectrophotometer (UNICO Model 1200).

Decolourization rate was expressed as percentage decolourization and calculated using the formula:

$$\% \text{ Decolourization} = \frac{A - B}{A} \times 100$$

Where A is initial absorbance and B final absorbance.

Degradation Analysis:

Microbial treatment was carried out on the effluent for 7 days after which it was centrifuged. The cell free supernatant was treated with dichloromethane and the organic layer was used for the degradative analysis. GC-MS analysis was performed using a QP2010 mass spectrometer from Shimadzu (Kyoto, Japan) fitted with a GC-17A gas chromatograph (Shimadzu; Kyoto, Japan). The ionization voltage was 70 eV. Gas chromatography was conducted in the temperature-programming mode with a HP5MS column (30 m by 0.25 mm ID by 0.25µm) from Restek (Bellefonte, PA). The initial column temperature was held at 60°C for one minute, then increased linearly to 250°C at 10°C/min, and held for five minutes at 250°C. The temperature of the injection port was 250°C and the GC/MS interface was maintained at 300°C. Helium was used as carrier gas with a flow rate of 1.0 ml/min. Injection was splitless to increase sensitivity. Identification of degradation products was made by comparison of retention time and fragmentation pattern with known reference compounds as well as with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS.

Cytotoxicity Test:

Cell free supernatant was used to conduct cytotoxicity test according to the modified method of Meyers *et al.* (1982) using brine shrimp cysts. The lethality of the untreated and treated samples of effluents was calculated from the mean survival of larvae in the samples using Finney's Computer software to determine the LC<sub>50</sub> of each sample.

### 3. Results

Thirteen bacteria were isolated from the effluent from local textile industries and soil where effluent had been discharged and they were identified. Majority of the bacterial isolates were *Bacillus sp.* *Bacillus licheniformis* had the highest decolourization ability on the effluent having 43.23% decolourization potential.

Figure 1 shows the percentage of decolourization of effluents and dyes (army green and Gambia gold) by the *B. licheniformis*. *B. licheniformis* was able to decolourize the dyes i.e. army green (63.76%) and Gambia gold (90.32%) more than it could decolourize the effluents (43.23%). *B. licheniformis* was able to decolourize dye effluent more than all other isolates, hence its cell free supernatant was used for the degradation and detoxification analysis.

Table 1 shows the compounds present in effluent samples before and after bacterial treatment using *Bacillus licheniformis* as revealed by GC-MS analysis. The untreated effluent had a total of twenty nine compounds, some of which were totally degraded during microbial treatment whereas the concentration of some compounds was only reduced during the microbial treatment. For example, the untreated effluent contains compounds such as 1-methylisoquinoline, 2-Naphthalenamine, 7-methylquinoline, 4-methylquinoline, 2,7-dimethylquinoline, 2,4-dimethylquinoline, Dodecane, 2-methyldodecane, N, N- dimethyl-1,1-naphthalenamine, N-ethyl-1-Naphthalenamine, 2-methyl-1-Naphthalenecarboxylaldehyde, Ethyleneglycol monododecyl ether, 9,10-Anthracenedione, 2- hexyl-1-Decanol, n-dodecyl glycidyl ether, Acetate fast orange, Oleic acid amide and 2-amino-9,10-Anthracenedione which were totally removed in the effluent treated by *B. licheniformis*. Some new compounds formed by *B. licheniformis* during the treatment of effluent include, Octadecane, Eicosane, Dotriacontane etc. It was observed that during the treatment of effluents using *B. licheniformis*, most cyclic hydrocarbons such as Quinoline, 2-methylquinoline etc was broken down into straight chain hydrocarbons such as hexadecane, heptadecane etc.

For cytotoxicity test, the higher the LC<sub>50</sub>, the lower the toxicity of such sample. *B. licheniformis* treated effluent had reduced toxicity compared to the untreated effluent. *B. licheniformis* treated effluent had an LC<sub>50</sub> of 642.72 whereas the untreated effluent had an LC<sub>50</sub> of 1.61.

**TABLE 1: Compounds present in the effluent before and after treatment with *B. licheniformis*.**

Peak number	Name of compound	Percentage of compound present in the untreated sample	Percentage of compound present in the treated sample	Molecular formula	Molecular mass
1	1,2,3,4- tetramethyl benzene	1.2	0	C <sub>10</sub> H <sub>14</sub>	134
2	1,2,4,5-tetramethyl benzene	0	1.98	C <sub>10</sub> H <sub>14</sub>	134
3	1-ethyl-3,5-dimethyl benzene	0.82	1.55	C <sub>10</sub> H <sub>14</sub>	134
4	Naphthalene	1.25	2.11	C <sub>10</sub> H <sub>8</sub>	128
5	4,6-dimethyl dodecane	3.74	8.02	C <sub>14</sub> H <sub>30</sub>	198
6	Quinoline	19.74	2.85	C <sub>9</sub> H <sub>7</sub> N	129
7	2-methylquinoline	6.17	1.04	C <sub>10</sub> H <sub>9</sub> N	143
8	2-Naphthalenamine	1.25	0	C <sub>10</sub> H <sub>9</sub> N	143
9	1-methylisoquinoline	1.24	0	C <sub>10</sub> H <sub>9</sub> N	143
10	7-methylquinoline	5	0	C <sub>10</sub> H <sub>9</sub> N	143
11	4-methylquinoline	4.29	0	C <sub>10</sub> H <sub>9</sub> N	143
12	2,7-dimethylquinoline	2.35	0	C <sub>11</sub> H <sub>11</sub> N	157
13	2,6-dimethylquinoline	0	0.7	C <sub>11</sub> H <sub>11</sub> N	157
14	2,4-dimethylquinoline	2.43	0	C <sub>11</sub> H <sub>11</sub> N	157
15	Indole	0	1.16	C <sub>8</sub> H <sub>7</sub> N	117
16	Dodecane	2.84	0	C <sub>12</sub> H <sub>26</sub>	170
17	Octadecane	0	4.79	C <sub>18</sub> H <sub>38</sub>	254
18	N,N-dimethyl-1-Naphthalenamine	1.1	0	C <sub>12</sub> H <sub>13</sub> N	171
19	2- methyl dodecane	2.1	0	C <sub>13</sub> H <sub>28</sub>	184
20	N-ethyl-1-Naphthalenamine	0.72	0	C <sub>12</sub> H <sub>13</sub> N	171
21	2-methoxy-1-Naphthalenecarboxaldehyde	1.26	0	C <sub>12</sub> H <sub>10</sub> O <sub>2</sub>	186
22	Tetracosane	6.78	13.04	C <sub>24</sub> H <sub>50</sub>	338
23	Hexadecane	2.02	14.53	C <sub>16</sub> H <sub>34</sub>	226
24	Heptadecane	3.93	13.35	C <sub>17</sub> H <sub>36</sub>	240
25	2,5,5-trimethylheptane	0	0.31	C <sub>10</sub> H <sub>22</sub>	142
26	Eicosane	0	8.25	C <sub>20</sub> H <sub>42</sub>	282
27	Eicosanoic acid	0	0.5	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312
28	Ethylene glycolmonododecyl ether	2.78	0	C <sub>14</sub> H <sub>30</sub> O <sub>2</sub>	230
29	9,10-Anthracenedione	6.2	0	C <sub>14</sub> H <sub>8</sub> O <sub>2</sub>	208
30	2-hexyl-1-Decanol	3.27	0	C <sub>16</sub> H <sub>34</sub> O	242
31	Nonacosane	3.97	0	C <sub>29</sub> H <sub>60</sub>	408.79
32	n-dodecyl glycidyl ether	3.51	0	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242
33	Oleic acid amide	3.02	0	C <sub>18</sub> H <sub>35</sub> NO	281
34	Dotriacontane	0	5.07	C <sub>32</sub> H <sub>66</sub>	450
35	Tetratetracontane	0	0	C <sub>44</sub> H <sub>90</sub>	619
36	Tetracosane	4.32	11.13	C <sub>24</sub> H <sub>50</sub>	338
37	Tetrapentacontane	0	0	C <sub>54</sub> H <sub>110</sub>	758
38	Tetradecanal	0	1.66	C <sub>14</sub> H <sub>28</sub> O	212
39	Acetate fast orange	1.87	0	C <sub>12</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>	242
40	2-amino-9,10- Anthracenedione	0.84	0	C <sub>15</sub> H <sub>11</sub> NO <sub>2</sub>	237
41	(Z)-9-Octadecenamide	0	4.55	C <sub>18</sub> H <sub>35</sub> NO	281
42	Octanoic acid pentadecyl ester	0	1.32	C <sub>17</sub> H <sub>34</sub> O	254
43	O,O-diethyl O-Phosphorothioic acid	0	2.06	C <sub>9</sub> H <sub>11</sub> C <sub>13</sub> NO <sub>3</sub>	349
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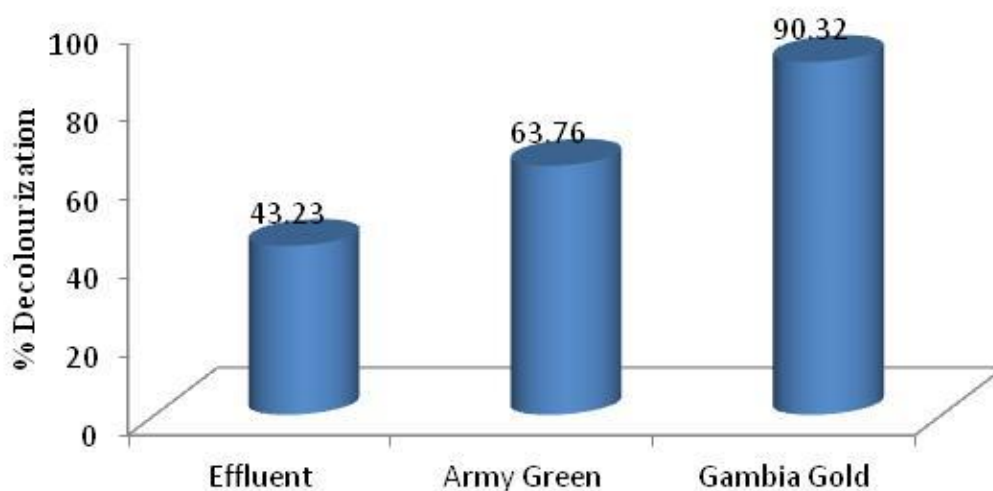


Figure 1: Decolourization of effluent and dyes by *Bacillus licheniformis*

#### 4. Discussion

Textile effluent collected from local textile industries in Itoku, situated in Abeokuta was highly coloured. It was also observed that the effluent was highly odorous. This is in agreement with previous findings regarding textile effluents (Faryal and Hameed, 2005) who reported that effluents from textile industries are highly colored and has a foul smell.

There has been earlier reports on the upsurge in the search for cost effective and environmentally friendly alternatives to the conventional methods of treating wastes in the environment (Asamudo *et al.*, 2005). A number of microorganisms have been studied to unfold their degradative abilities in remediation of pollutant (Melvin, 2006). In this study, thirteen bacterial isolates were isolated. Majority of the bacterial isolates were of the genera *Bacillus*. This is similar to earlier studies carried out by Arun-Prasad and Bhaskara-Rao (2010) in which they isolated *Bacillus sp* from textile effluent. Also in another study carried out by Murugalatha *et al.* (2010), he treated textile effluent using *Bacillus* species isolated from processed food. The ability of microbial isolates to achieve a high percentage of decolourization on single dyes has been reported in previous studies (Chaube *et al.*, 2010). Similar work done by Murugalatha *et al.* (2010) using *Bacillus sp* isolated from processed food to treat effluents from textile industries, he observed decolourizing activities ranging from 40.74-47.73% even after 14 days. The ability of the isolates to achieve high decolourization percentage in the dyes (army green and Gambia gold) as compared to the rate at which they decolourized the effluents and the combination of dyes may be attributed to the components making up each of the dyes and effluents. Army green was a

combination of black and green dye, Gambia gold was a combination of red and brown dyes. The ability of microbial isolates to achieve high percentage of decolourization on single dyes has been reported in previous studies (Chaube *et al.*, 2010).

Dyes used in textile industries are usually toxic (Ogunjobi *et al.*, 2012), since the dyes form a major component of the effluent from local textile industries, it makes the effluent to be toxic. The toxicity study revealed that there was a decrease in the toxicity of the treated sample compared to the untreated effluent samples. This result is similar to those obtained by Bergsten-Torralba *et al.* (2009) in which *P. simplicissimum* reduced efficiently the toxicity of a dye (RB21) from moderately acutely toxic to minor acutely toxic.

In conclusion, since the isolate obtained in this study was able to decolourize the effluent and dyes and it could detoxify and degrade it, it can be employed in the treatment of effluent from local textile industries in Nigeria. It can also be used with other microorganisms which have decolourization ability to achieve higher decolourization, degradation and detoxification of the dye effluents and dyes.

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