Biodegradation of Pollutants in Waste Water from Pharmaceutical, Textile and Local Dye Effluent in Lagos, Nigeria

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Introduction

Microorganisms have been utilized in environmental remediation for decades. Bioremediation is defined as the use of biological agents such as bacteria, fungi, or green plants (phytoremediation) to remove or neutralize hazardous substances in polluted soil or water. Increasing human population has led to an increase in industrial activities. One of the main sources of pollution worldwide is the textile industry and its dye-containing wastewaters. About 25% of textile dyes are lost during the dyeing process, and 2-20% are discharged as aqueous effluents in different environmental components. The discharge of dye-containing effluents into the water environment is undesirable because of its colour, direct release and its breakdown products are toxic, carcinogenic or mutagenic to life forms due to carcinogens such as benzidine, naphthalene and other aromatic compounds.

Background. Discharged effluents from industry have been responsible for the deterioration of the aquatic environment in many parts of the world, especially in developing countries. Increasing industrialization and urbanization have resulted in the discharge of large amounts of waste into the environment, resulting in high pollution loads. Utilization of microbes such as fungi and bacteria have been used for pollution degradation.

Objectives. The aim of this research was to utilize microbial agents such as fungi and bacteria to reduce pollutant loads such as heavy metals in effluent samples.

Methods. Three types of effluent (pharmaceutical, textile effluent, and dye) were obtained from Surulere in Lagos Metropolitan Area, Nigeria. Heavy metals analysis was carried out using a flame atomic adsorption spectrophotometer according to standard methods. Samples were cultured for microbes and identified. Bacteria samples were inoculated on nutrient agar and incubated at 37°C for 24 hours. Fungi counts were carried out using potato dextrose agar and incubated at 28°C for 3-5 days. The isolated organisms were identified based on their morphological and biochemical characteristics. Then 100 mL of the effluents was dispensed into 250 mL flasks, and the pH of the medium was adjusted to 7.2 by the addition of either sodium hydroxide or hydrogen chloride and autoclaved at 121°C for 15 minutes. The autoclaved flask was inoculated with 1 mL of bacteria and fungi for 21 days and pH was recorded properly every 48 hours.

Results. The results of the physicochemical parameters indicated that conductivity, total suspended solids, total dissolved solids, turbidity, chemical oxygen demand and biochemical oxygen demand for all the three industrial effluents were higher than the World Health Organization (WHO) permissible limits. Heavy metal analysis results show that the effluents had high values for cadmium, above the WHO limit of 0.003 mg/L. Concentrations of zinc ranged from 0.136-1.690 mg/L, and nickel ranged between 0.004-0.037 mg/L for the three effluents, within the WHO limit. The identified bacteria were Bacillus subtilis, Klebsiella pneumonia, Salmonella typhi and Bacillus cereus and isolated fungi were Aspergillus flavus and Penicillium chrysogenum. All the physicochemical parameters and heavy metal concentrations were reduced after the biodegradation study in the effluents.

Conclusions. The responses observed in the various microbes indicated that the use of microbes for the reduction of environmental pollutants has an advantage over the use of other methods because it is environmentally friendly, low cost, and no new chemicals are introduced into the environment. This method should be encouraged for pollution reduction to bring about ecosystem sustainability advocated for Ghana.

Competing Interests. The authors declare no competing financial interests.

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Environmental problems such as appearance of colour in discharges from various industries, combined with the increasing cost of water for the industrial sector, have made the treatment and reuse of effluent increasingly attractive to the industry.

The textile industry is one of the oldest industries in India with over 1000 factories. Due to the volume and composition of its effluent, textile wastewater is considered to be the most polluting among all of the industrial sectors. Wastewater from a typical textile plant is characterized by high values of biochemical oxygen demand (BOD), chemical oxygen demand (COD), colour and pH. It is a complex and highly variable mixture of many polluting substances ranging from inorganic compounds and elements to polymers and organic products. The incomplete use of dye and washing operations result in textile wastewater retaining a considerable amount of dye.

Pharmaceutical wastewater is a complex mixture of different organic and inorganic compounds, including residues of active pharmaceutical substances, solvents, and toxic and bio-recalcitrant chemicals that inhibit microbial activity of the activated sludge process and present a great challenge for the proper treatment and downstream processing of wastewater. In the pharmaceutical industry, wastewater is mainly generated through equipment washing activities. Although the wastewater discharged is small in volume, it is highly polluted due to the presence of substantial amounts of organic pollutants. Levels of wastewater pollution vary from industry to industry, depending on the type of process and the size of the industry. Typically, pharmaceutical wastewater is characterized by a high COD concentration and some pharmaceutical wastewaters have COD levels reaching as high as 80,000 mg/L. Pharmaceutical companies are one of the major contributors of hazardous and toxic effluents. Ireland alone generates about 43 tons BOD in its pharmaceutical industry. The recycling of treated wastewater has been recommended due to the high levels of contamination stemming from dyeing and finishing processes (i.e. dyes and their breakdown products, pigments, dye, intermediate, auxiliary chemicals and heavy metals).

The aim of this research was to utilize microbial agents such as fungi and bacteria to reduce pollutant loads such as zinc, cadmium, and nickel in effluent samples.

**Methods**

**Sampling Location**

Effluents were collected in Surulere in Lagos Metropolitan Area, Nigeria from different industries from their main sites. Surulere is a commercial area where many manufacturing industries are located. The coordinates of the sample locations are presented in Table 1. Lagos Metropolitan Area is a megacity and contains 70% of the industries of Nigeria.

**Sample Collection**

Effluent samples were collected at the discharge pipe at about 7:30 am with three replicates (A1, A2, A3, etc.) from pharmaceutical, textile and dye industries. The samples were collected in sterile sample bottles and transported immediately to the laboratory and stored at around 4°C. Then, 250 mL samples were collected and put in sterile reagent bottles (500 mL capacity). The samples were subjected to immediate physicochemical analysis on site. These samples served as the source for the isolation of micro-organisms.

**Physicochemical and Heavy Metal Analysis of Effluents**

All samples were analyzed for heavy metals (zinc, cadmium, and nickel) and physicochemical parameters according to internationally accepted procedures and standard methods. The analyzed parameters included temperature, chemical oxygen demand, dissolved oxygen, biochemical oxygen demand, turbidity, odour, colour, total suspended solids, pH, conductivity, and total dissolved solids. In addition, pH, temperature, and dissolved oxygen were determined on site using appropriate meters (pH meter Hanna HI9813, TDS-3

| Industry | GPS Readings |
|----------|--------------|
| Pharmaceutical | Longitude N06° 31.22, Latitude E003° 14.15 |
| Textile | Longitude N06°29.135, Latitude E003° 21.232 |
| Dye | Longitude N06°29.396, Latitude E003° 19.960 |

**Table 1 — Coordinates of the Sample Locations**
HM digital for temperature, and DO analyser model JPSJ-605). The concentrations of heavy metals were determined using an atomic absorption spectrophotometer.

**Microbial Analysis**

**Total Bacterial Count**

The collected samples were analysed for the presence of microorganisms. First, 1 mL of each effluent sample was transferred into 9 mL of sterile saline solution in a test tube and shaken vigorously. The solution was serially diluted and 10⁻³ dilution was taken and plated using the pour plate technique on Petri dishes. The bacteria were inoculated on nutrient agar and incubated at 37°C for 24 hours. This was carried out using procedures which have been previously reported.²¹

**Total Fungal Count**

Fungal counts were conducted using potato dextrose agar with 10% tartaric acid using the spread plate method. This was carried out according to previously reported methods.²² Microbial count of the effluents samples were reported as colony forming units per gram (cfu/g).

**Characterisation and Identification of Organisms**

The identification of bacteria was based on biochemical characterizations including citrase, urease, catalase, indole, raffinose, xylose, galactose, starch hydrolyses, and oxidase reaction. The macroscopic colonial appearances of fungal growth in plates were observed and recorded. The macroscopic examinations were based on colony texture, size, pigmentation, time of growth, color on the reverse side of the plate and colony margin.²³ A drop of lactophenol cotton blue was placed on a grease free, scratch-free glass slide.²⁴ A small portion of the fungal growth was picked with a wire loop and teased out using a mounting needle. The preparation was covered with a cover slip.²⁵ The slide was observed under 10x and 40x objective lenses. Observed characteristics were recorded and compared with the established identification keys as previously described.²⁶

**Biodegradation of Effluents**

**Bacteria**

Mineral salt medium prepared with the following composition was used for the studies: disodium phosphate (1.065 g) ammonium chloride (0.25 g), magnesium sulfate heptahydrate (0.10 g), monopotassium phosphate (0.65 g), and added to 500 mL of the effluents. Then, 100 mL of the effluents was dispensed into 250 mL flasks, the pH of the medium was adjusted to 7.2 by addition of either sodium hydroxide or hydrogen chloride, and autoclaved at 121°C for 15 minutes. The autoclaved flask was inoculated with 1 mL of bacteria inoculum of the microorganism and the flask was incubated for 21 days. The pH was recorded every 48 hours.

**Fungi**

Mineral salt medium prepared with the following composition was used for the studies: disodium phosphate (1.065 g), ammonium chloride (0.25 g), magnesium sulfate heptahydrate (0.10 g), monopotassium phosphate (0.65 g), and added to 500 mL of the effluents. Then, 100 mL of the effluents was dispensed into 250 mL flasks, the pH of the medium was adjusted to 5.6 by addition of either sodium hydroxide or hydrogen chloride, and chloramphenicol was added and autoclaved at 121°C for 15 minutes. The fungi plate was emulsified with 10 mL of sterilized distilled water, and then 1 mL of the fungal inoculum was inoculated into each autoclaved flask. The flasks were kept in the mechanical shaker and incubated at room temperature for 21 days. The pH was recorded at 3-day intervals.

**Results**

**Physicochemical Parameters of Effluents**

The results of the physicochemical parameters show that conductivity, total suspended solids (TSS), total dissolved solids (TDS), turbidity, COD and BOD for all the three industrial effluents were higher than the World Health Organization (WHO) permissible limits for water quality.²⁷ The effluent from the local dye industry had the highest values for pH (12.02), conductivity (24500 mS cm⁻¹), DO (10 mg/L), TSS (7100 mg/L), TDS (12.500 mg/L), COD (290 mg/L), and BOD (150 mg/L), compared to effluents from the other two industries.

The pH of pharmaceutical and textile effluents were within the WHO permissible limits.²⁷ Physical observation revealed the colour of pharmaceutical effluent, textile effluent and local dye effluent to be yellowish, black and reddish-brown, respectively. Odour was observed to be choky for pharmaceutical effluent and pungent smell was observed for textile and local dye effluents. Heavy metal analysis results show that the effluents had high values for cadmium, above the WHO limit of 0.003 mg/L. The concentration of zinc ranged from 0.136-1.690 mg/L, and the concentration of nickel ranged from 0.004-0.037 mg/L for the three effluents, all within the WHO limit.
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### Parameters

| Parameters                      | Pharmaceutical effluent | Textile effluent | Local dye effluent | WHO or Standard Organisation of Nigeria (SON) Federal Environmental Protection Agency - Nigeria (FEPA) permissible limits |
|---------------------------------|--------------------------|-----------------|-------------------|---------------------------------------------------------------------------------------------------------------------|
| pH                              | 6.00                     | 6.89            | 12.02             | 6.5-8.5 [27, 28]                                                                                                        |
| Temperature (°C)                | 27.31                    | 26.72           | 26.4              | <35 [27, 28]                                                                                                          |
| Conductivity (µScm⁻¹)           | 12900                    | 6680            | 24500             | 200 [27, 28]                                                                                                          |
| DO (mg/l)                       | 8.17                     | 5.03            | 10                | 8.5 [27, 28]                                                                                                          |
| Turbidity (Formazin Turbidity Unit) | 800                      | 795             | 3550              | —                                                                                                                      |
| TSS (mg/L)                      | 1000                     | 1400            | 7100              | >10 [27, 28]                                                                                                          |
| TDS (mg/L)                      | 8290                     | 4250            | 12500             | 500 [27, 28]                                                                                                          |
| COD (mg/L)                      | 125                      | 109             | 290               | 10 [27, 28]                                                                                                          |
| BOD (mg/L)                      | 60                       | 51              | 150               | 2.5 [27, 28]                                                                                                          |
| Zinc (mg/L)                     | 0.139                    | 0.136           | 1.690             | 3.0 [29]                                                                                                              |
| Cadmium (mg/L)                  | 0.337                    | 0.183           | 0.030             | 0.003 [27, 28]                                                                                                         |
| Nickel (mg/L)                   | 0.004                    | 0.037           | 0.012             | 0.02 [27, 28]                                                                                                         |
| Colour                          | Yellowish                | Black           | Reddish brown     | —                                                                                                                      |
| Odour                           | Choky                    | Pungent         | Pungent           | —                                                                                                                      |

### Table 2 — Characterization of the Three Wastewater Samples Compared to Permissible Limits

- **Abbreviations**: DO, Dissolved oxygen; TSS, Total suspended solids; TDS, Total dissolved solids, a-World Health Organisation standard, b-Federal Environmental Protection Agency.

### Table 3 — Biochemical Test of Bacterial Isolates

- **Abbreviations**: GPR, Gram positive rod; GNR, Gram negative rod; A, Pharmaceutical effluent; B, Textile effluent; C, Dye effluent.
Biochemical Identification of Bacterial Isolates

The results of the biochemical test are presented in Table 3. The identified bacteria included Bacillus subtilis, Klebsiella pneumonia, Salmonella typhi and Bacillus cereus. The isolates were Gram-positive rod for all samples from pharmaceutical and local dye effluent, and Gram positive and Gram negative rod for samples from textile effluent. Isolates were all negative for urease for samples from pharmaceutical effluent and negative for hydrogen sulfide gas production and indole for all samples from the three effluents.

Morphological Characteristics and Identities of Isolated Fungi Associated with Biodegradation

The isolated fungi were Aspergillus flavus and Penicillium chrysogenum. The colony morphology and microscopic characterization are presented in Table 4.

Biodegradation

Bacteria

The microorganisms used for biodegradation include Bacillus subtilis for sample A (pharmaceutical effluent), Salmonella typhi for sample B (textile effluent) and Bacillus cereus for sample C (Dye effluent). There was a reduction in pH of the three effluents after 21 days. The pH of the pharmaceutical effluent reduced from 7.20 – 6.70, pH of the textile effluent reduced from 7.20-6.94, and pH of the dye effluent reduced from 7.20 – 7.00 after 21 days.

Results of the physicochemical analysis and heavy metal concentrations of the three effluents before and after biodegradation are presented in Table 5. All the physicochemical parameters and heavy metal concentrations were reduced after the biodegradation study (Table 5). Reductions of 18.41%, 9.0%, and 32.00% were recorded for conductivity in the pharmaceutical, textile and dye effluent, respectively. There was a 55.94%, 32% and 62% reduction in dissolved oxygen in the pharmaceutical, textile and dye effluent, respectively.
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A reduction of 13.75%, 11%, and 29% was recorded for turbidity in pharmaceutical, textile and dye effluent respectively. There was a reduction of 38%, 82.5% and 26% for total suspended solids in the pharmaceutical, textile and dye effluent, respectively. Total dissolved solids value showed a 62%, 28% and 33% reduction in pharmaceutical, textile, and dye effluent, respectively. A reduction of 51%, 43.05%, and 78% was recorded for COD in the pharmaceutical, textile and dye effluent, respectively. A reduction of 46%, 35%, and 77% was recorded for BOD in the pharmaceutical, textile and dye effluent, respectively. There was a reduction of 63%, 48%, 53% for cadmium, 50%, 56%, 58% for nickel, and 41%, 20%, 36% for zinc in pharmaceutical, textile and dye effluents, respectively.

**Fungi**

The microorganism used for biodegradation was *Penicillium chrysogenum*, since it is common to all three effluents. There was a reduction in the pH of the three effluents after 21 days. The pH of the pharmaceutical effluent reduced from 5.6-5.33, the pH of the textile effluent reduced from 5.60-4.92, and the pH of the dye effluent reduced from 5.60-5.26 after 21 days.

| Parameters                     | Pharmaceutical effluent | Textile effluent | Dye effluent |
|--------------------------------|-------------------------|-----------------|--------------|
| **pH**                         | Before: 6.0              | After: 6.67     | Before: 12.0 | After: 6.68 |
| **Temperature (ºC)**           | 27.31                   | 25.0            | 26.40        | 25.40       |
| **Conductivity (µScm⁻¹)**       | 12900                   | 10525           | 24500        | 16650       |
| **DO (mg/L)**                   | 8.17                    | 3.6             | 10           | 3.8         |
| **Turbidity (Formazin Turbidity Unit)** | 800                     | 690             | 3550         | 2500        |
| **TSS (mg/L)**                  | 1000                    | 620             | 7100         | 5200        |
| **TDS (mg/L)**                  | 8290                    | 3110            | 12500        | 8325        |
| **COD (mg/L)**                  | 125                     | 61              | 290          | 63          |
| **BOD (mg/L)**                  | 60                      | 32              | 150          | 34          |
| **Cadmium (mg/L)**              | 0.337                   | 0.124           | 0.030        | 0.014       |
| **Nickel (mg/L)**               | 0.004                   | 0.002           | 0.012        | 0.005       |
| **Zinc (mg/L)**                 | 0.139                   | 0.082           | 1.690        | 0.940       |

**Figure 2 — Graph of pH for the three samples after fungi degradation**

Abbreviations: DO, Dissolved oxygen; TSS, Total suspended solids; TDS, Total dissolved solids

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Discussion

In this study, the results of the physicochemical analysis of the three effluents showed levels of almost all the analyzed parameters to be higher than WHO permissible limits. This agreement with a report by Lokhade et al., which stated the physicochemical parameters of effluent was greatly increased in the paint, pharmaceutical and dye industry effluent compared with permissible limits. Heavy metal analysis in the three samples revealed the presence of cadmium, nickel and zinc, which may be because that these metals form part of the chemical constituents of various mixtures used as by-products in these industries. Similar findings were also reported by Anyakora et al., who stated that the concentration of different metals in the effluent varied significantly, giving credence to the idea that these metals are part of the manufacturing process. Cadmium was detected in the highest concentration in the sample of pharmaceutical effluent. The concentration of nickel was also revealed to be the highest in the textile effluent, while local dye effluent contained Penicillium chrysogenum, Bacillus subtilis and Bacillus cereus. The pharmaceutical effluent was biodegraded using Bacillus subtilis and Penicillium chrysogenum, and a reduction in value of physicochemical parameters and heavy metals concentration was observed. Biodegradation of the textile effluent by Salmonella typhi and Penicillium chrysogenum showed a reduction in the initially recorded values of physicochemical parameters and heavy metals. This shows that the bacteria and fungi used were capable of breaking down and utilizing these pollutants with low or no impact on the various components of the aquatic environment. These results are in agreement with those of Joutey et al., who reported that microbes that inhabit the soil and groundwater utilize some pollutant chemicals for food and when they completely digest the chemicals and change them into water and harmless gases. The reduction of the physicochemical values by the isolated microbes may be due to consumption of inorganic and organic matter by microbes for food, a conclusion supported by the work of Elizabeth et al. and Noorjahan and Jamuna.

Conclusions

Most industrial effluents contain hazardous chemicals that may have direct or indirect impacts on aquatic biota by bioaccumulation along the food chain and which may later become magnified. Many heavy metals that are found in these effluents have been shown by previous studies to be toxic to both aquatic fauna and flora and therefore stricter regulation of these industries is needed. This present study confirmed the capability of different microbes (Bacillus subtilis, Salmonella typhi and Bacillus cereus) to break down the pollutants in three effluents, pharmaceutical, textile and local dye, to a less toxic form. These microbes should be enhanced in their natural ecosystem in order to be able to degrade more of these pollutants. This method should be embraced because of its advantage over other methods; it is environmentally friendly, lower cost, equally effective, and able to bring about a cleaner and more sustainable ecosystem.

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Research

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