E-ISSN 2656-8039
INTERNATIONAL JOURNAL OF
ENVIRONMENT, ENGINEERING & EDUCATION
VOL. 4, NO. 3, 2022

www.ijeedu.com

Research Article

Physico-Chemical and Heavy Metal Valences Reduction of Waste Water from The Beverage Industry by Fungi (Penicillium Sp.)

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Received: *September 29, 2022;* ***Accepted:*** *December 22, 2022;* ***Published:*** *December 27, 2022*

Abstract: This work aimed to characterize beverage wastewater generated in the beverage industry and to assess wastewater treatment plant performance by fungi (Penicillium sp.) and the feasibility of wastewater reuse. Freshly discharged beverage wastewater was collected and analyzed for the physicochemical parameters such as Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solid (TDS), Nitrate, Phosphate, Magnesium, Calcium, Iron, Copper, and Zinc by standard methods. At a 7-day interval, 190ml of the sterilized local dye wastewater was inoculated with Penicillium sp. for two weeks, and the physicochemical parameters were determined. The results observed for raw, bio-treated and removal efficiency showed: BOD (280 mg-1, 255 mg-1 , 108 mg-1 and 19.64 %, 61.43 %); COD (540 mg-1, 420 mg-1, 285 mg-1 and 43.75%, 88.13%); Nitrate (130 mg-1, 90 mg-1, 25 mg-1 and 30.77%, 80.77%); Phosphate (48 mg-1, 25 mg-1, 5 mg-1 and 30.91%, 78.18%); Calcium (55 mg-1, 38 mg-1, 12 mg-1 and 30.91%, 78.18%); Iron (29 mg-1, 18 mg-1, 07 mg-1 and 37.93 %, 75.86 %); Copper (0.09 mg-1, 0.07 mg-1, 0.02 mg-1 and 22.22 %, 77.78 %); Zinc (0.08, 0.06, 0.03 mg-1 and 22.5%). Fungi (Penicillium sp.) demonstrated the ability to remove pollutants and other wastes from beverage wastewater. These results indicate that some companies employing treatment methods for their effluents do not remove the parameters and heavy metals.

Keywords: Environmental Issues; Health Hazards; Industrial Activities; Water Pollution; Wastewater Treatment.

1. Introduction

Industrial liquid effluents are one of the principal sources of heavy metals responsible for environmental pollution [1]. The current scenario of a sustainable environment is highly vulnerable since most waterborne waste discharges from domestic and industrial sources are channeled into natural water bodies [2]. In recent years, large-scale usage of chemicals in various human activities has grown considerably, and pollution has assumed an escalating dimension due to the continual expansion of urbanization, industrial development, and agricultural activities [3]. Water pollution by industrial effluent is one of the vital issues of environmental concern today [4].

One of the most concerning environmental issues today derives from the contamination of various environmental components (soil, water, and air), which triggers ecological and anthropological health hazards because of exposure to toxic levels of a variety of substances [5], [6]. Industrial advances have been

This article citation: K. A. Lawal, A. A. Abideen, I. K. Lawal, O. A. Owolabi, K. F. Bamidele, M. A. Oluwagbemiga, "Physico-Chemical and Heavy Metal Valences Reduction of Waste Water from The Beverage Industry by Fungi (Penicillium Sp.)," Int. J. Environ. Eng. Educ., vol. 4, no. 3, pp. 1-4, 2022.
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associated with an increase in the introduction of pollutants into the environment [7]. Among these, water contamination by metals is noteworthy.

Heavy metals are non-degradable, persistent in the environment, and show a high dispersal capacity by water and a considerable bioaccumulation rate in plants [8], fish [9], other animals, and humans [10]. Given the proper conditions, they can accumulate toxic levels [11]. Their transfer via the food web to humans is a real risk; therefore, scientific and technological investigations to find solutions to this dilemma are very appropriate [12], [13]. Heavy metals (HMs) usually reach the environment via industrial activities, agricultural management practices, and inappropriate waste disposal [11]. Although humans, as well as all living organisms, need variable quantities of some HMs, such as iron, zinc, copper, and chromium, for proper growth and development.

Heavy metals can be toxic in high concentrations [14]. HMs can enter the plant, animal, and human tissues through inhalation, consumption, and dermal contact, causing harmful effects [15]. The widespread contamination and toxicity of HMs to biota, particularly the native microbial community, plays a vital role in ecosystem preservation through nutrient cycling and contaminant removal [16]. Therefore, it is essential and urgent to explore efficient and economical ways for HM remediation to protect the environment [17].

The world's population is growing and has an impact on the environment. As the population grows, the demand for natural resources, including water, also grows. This means population growth directly affects the quality of the water supply, and water problems have grown with the population [18]. In addition, industrial and urban activities in developing countries have increased in recent years, contributing to increased water pollution [14]. Global water scarcity is caused by the physical scarcity of this resource and increasing water quality deterioration in many countries, which reduces the amount of safe water available for use [19].

For most people in developing countries, consuming contaminated water often negatively influences human health [20]. Also, in these countries, the effects of increased pollution are remarkably problematic as there is poor treatment of contaminated water [14]. Unfortunately, this vital resource is subjected to many contaminants, including heavy metals, considered one of the riskiest contaminants resulting from population growth and urbanization. Their environmental release has been going on for a lengthy period and is a continuing problem affecting billions' health [21], [22].

The separation of pollutants from waste streams has become a critical issue, and before releasing them into the environment, it is necessary to purify wastewater [23]. The disposal of untreated wastewater mainly causes water pollution by various industries [24]. Therefore, water pollution is a worldwide challenge in developed and developing countries [25]. As a result, interest has increased, and more comprehensive regulatory standards have been applied regarding releasing HMs and other pollutants into our natural environment [26].

Finally, according to the World Health Organization (WHO), providing safe water, sanitation, hygiene (WASH), and waste management is essential to protect human health and prevent infectious and transmissible disease outbreaks, such as the recent outbreak involving novel coronavirus disease (COVID-19) [27], [28]. Thus, wastewater treatment is necessary, especially under COVID-19 conditions, where water shortages can present an obstacle in many countries for purification and cleansing purposes.

The use of indicator bacteria such as fecal coliforms (FC) in water quality determination of freshwater sources is widely used [29]. Coliforms and Escherichia coli are highly important among bacterial indicators used in water quality definition and health risk assessment [30]. However, operational evaluation of the microbial load of wastewater (biologically) is often complicated because of variations in raw wastewater composition, strength, and flow rate owing to the changing and complex nature of the treatment processes [31]. Moreover, a lack of suitable processing variables limits the effective control of effluent quality [32].

Many problems in wastewater treatment that perform biological removal of pollutants are due to alterations in the microbial communities involved. Plate counting and most probable number (MPN) techniques have been used to study microbial communities in mixed culture systems. However, less than 1% of microorganisms in the environment can be cultivated by standard techniques because culture techniques fail to reproduce in artificial media, the niche of many microorganisms in high-diversity environments such as activated sludges [33]. Using heat-activated or dead biomass in industrial applications may offer some advantages over living cells, such as less sensitivity to heavy metals and excellent mechanical properties [34].

This study aims to analyze the microbial loads and heavy metal concentrations from the effluents of a food and beverage company in Nigeria to determine the degree of compliance of these industries with environmental laws. The research aimed to determine the level of some physicochemical parameters and heavy metals in the wastewater from beverages (YALE Foods Limited, Ring Road, Ibadan) after treatment with fungi (Penicillium Sp.)

2. Material and Methods

2.1. Sample Collection

Beverage wastewater samples were collected in clean laboratory containers from YALES FOOD LIMITED, Ibadan, and Oyo-state. The samples collected were then corked and transferred to the laboratory for analysis after 1-2 hours of sample collection. The organism employed in this study was a medical isolate of Penicillium Sp. obtained from the Department of Medical Microbiology and Parasitology, Osun State Teaching Hospital, Osogbo, Osun State.

2.2. Sterilization of Apparatus

All apparatus used in this study was thoroughly washed with detergent, rinsed with water, air-dried, and sterilized in a hot air oven at 160°C for two hours. Materials such as the mouth of the test tube, inoculating loop, and an inoculating needle were sterilized by flaming with a Bunsen burner before and after inoculation to prevent contamination.

2.3. Determination of Physicochemical Characteristics of Waste Water Samples

The physico-chemical parameters of wastewater from YALE FOODS, Ibadan, and Oyo-state were analyzed immediately using standard analytical procedures [35]. The physico-chemical parameters analyzed include biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids, nitrate, phosphorate, magnesium, calcium, zinc, and copper. The procedures involved in carrying out the physico-chemical processes are discussed below.

- *Biochemical Oxygen Demand*
  The organic matter in water was determined in terms of the oxygen required to oxidize it by treatment with potassium permanganate. In contact with oxidizable organic matter, potassium permanganate readily gives up its oxygen. The iodine formed dissolved more than potassium iodide and was estimated by titration with sodium thiosulfate using starch as an indicator.
- *Chemical Oxygen Demand (COD)*
  A predetermined amount of the reference substance dispersed in water was oxidized by potassium dichromate in a solid sulfuric acid medium with silver sulfate as a catalyst, under reflux for two hours. The residual dichromate was determined by titration with standardized ferrous ammonium sulfate. In the case of chlorine-containing substances, mercuric sulfate was added to reduce chloride interference.
- *Determination of Total Dissolved Solids*
  Total Dissolved Solids (TDS) for each water sample were determined using a TDS meter [35].
- *Determination of Nitrate*
  One test tube was filled to the 20ml mark with a sample, and one level spoonful (1.5 ml) of nitrates powder (containing 60% zinc dust and 40% barium sulphate) and one nitrate tablet (ammonium chloride) was added and shaken for a minute. The tube was allowed to stand for a minute and was inverted three or four times to aid flocculation. It was allowed to stand for two minutes to ensure complete settlement. The clear solution was dispersed into a 10 ml mark, and one Nitricol tablet (Sulfanilic acid, acting as the aromatic amine) was added, crushed, and mixed to dissolve. Then it was allowed to stand for 10 minutes for color development, and readings were taken on the Photometer (Wagtech) at 570 nm wavelength.
- *Determination of Phosphate*
  25 ml of the sample was added to 0.5 ml of ammonium molybdate and two drops of stannous chloride and mixed by swirling. A blue color developed within an hour and the intensity was measured using a spectrophotometer (21D) at 690 nm [35]. The concentration of the phosphate was calculated.

$$Phosphate = A - (B \times C) \quad (1)$$

where; Phosphate (mg/l); A = Absorbance of sample; B = Absorbance of blank sample; C = Volume of standard phosphate.

- *Determination of Magnesium*
  Ten ml of the sample was measured, a pinch of hydroxylamine hydrochloride was added, and 5 ml of mono-ethanol buffer (buffer 10) was added. Then two drops of Eriochrome black T indicator were added. This was titrated with 0.01 EDTA—the color changes from purple to blue-black.
- *Determination of Calcium*
  A ten-ml water sample was measured into a beaker; a pinch of potassium cyanide was added together with a pinch of hydroxylamine hydrochloride. Five ml of eight molar potassium hydroxides were added, and then a pinch of indicator (Putton and Reader reagent) was added and titrated with 0.01M EDTA using a burette. The color changes from brown to green.
- *Determination of Heavy Metals (Zinc and Copper)*
  The following heavy metals, Iron (Fe), Copper (Cu) and Zinc (Zn), were determined for each water sample using Test kits.

2.4. Experimental Set-Ups for conventional bioremediation of Beverages wastewater

To study the role of Penicillium sp. in beverages, the wastewater treatment method described by Adekanmi et al. [36] was employed where they treated slaughterhouse wastewater with microalgae for 14 days:

Wastewater from Beverage Processing + Penicillium Sp.

The experiments were conducted and incubated under the same conditions in a 250 mL Erlenmeyer flask for 7 and 14 days.

2.5. Inoculation and Sampling

10 mL of exponential growth of Penicillium sp. was inoculated into a 250 mL Erlenmeyer flask containing 190 ml of sterilized beverage wastewater Samples were taken for physicochemical analysis seven days after inoculation.

3. Result and Discussion

3.1. Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)

The Biochemical Oxygen Demand (BOD) recorded for raw beverage wastewater is found to be lower (255 and 108 mg/L) after 7 and 14 days of bio-treatment compared with the 280 mg/L obtained for raw beverage wastewater (Figure 1). They had reduction efficiencies of 19.64 and 61.43%) after 7 and 14 days of biotreatment with Penicillium Sp. (Figure 2).

The high degradation rate in week two (day = 14) could be because of the acclimatization of the microorganisms to the prevailing conditions; high organic matter present in pharmaceutical wastewater indicates higher BOD and COD. This conforms with the findings of Del Pozo et al. [37].

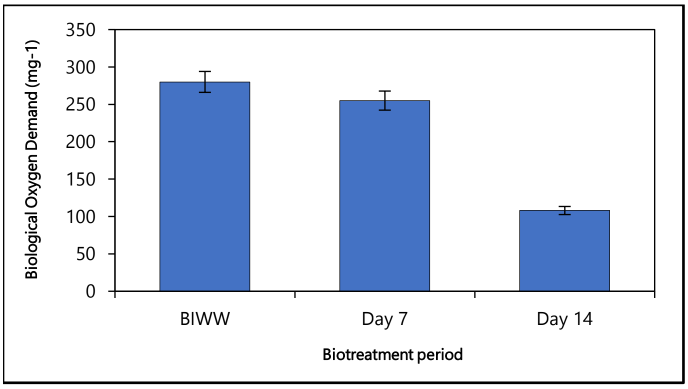

Figure 1. Biological Oxygen Demand of the Bio-treated Beverages Industry Wastewater with Penicillium Sp. after the 7th and 14th day of treatment (BIWW = Raw Beverages Industry Wastewater).

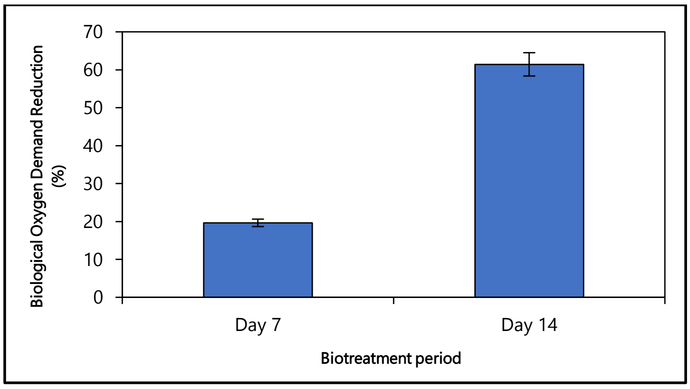

Figure 2. Biological Oxygen Demand Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

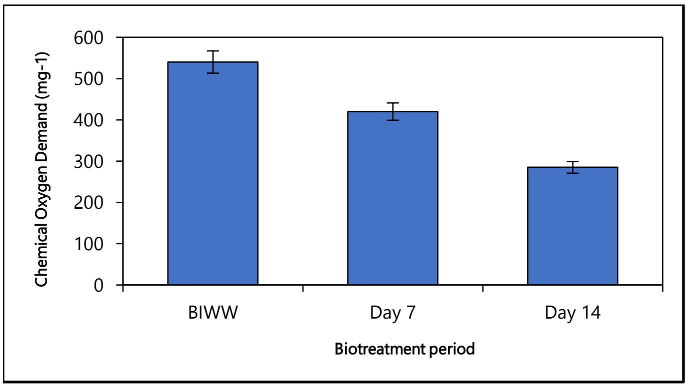

Figure 3. Biological Oxygen Demand Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

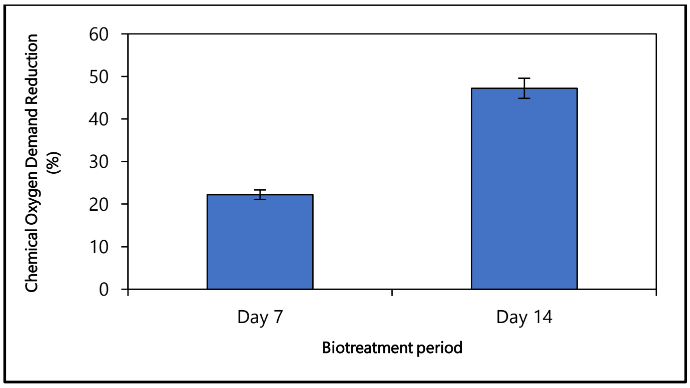

Figure 4. Chemical Oxygen Demand of the Bio-treated Beverages Industry Wastewater with Penicillium Sp. after the 7th and 14th day of treatment (BIWW = Raw Beverages Industry Wastewater).

The COD observed in this study showed that raw beverage wastewater was reduced to 420 and 285 mg/L, respectively, from an initial raw wastewater value of 540 mg/L after 7 and 14 days of treatment with Penicillium sp (Figure 3) at removal efficiencies of 22.22 and 47.22 %, respectively (Figure 4). The reduction rate of COD raw beverage wastewater confirms the effectiveness of the degradation process in reducing the pollutant load contained in the wastewater.

This fact significantly influenced the rest of the parameters and the nature of the waste. Some information on the wastewater biodegradability can be gained by comparing different measures; for example, BOD and COD, where a high ratio of BOD to COD shows a relatively high biodegradability, whereas a low ratio indicates that the wastewater is more slowly biodegraded [38].

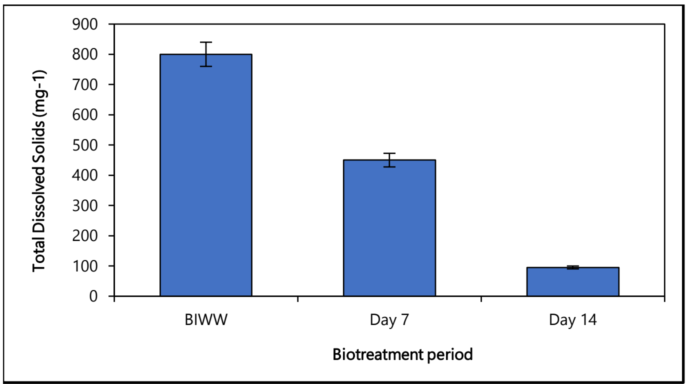

Figure 5. Total Dissolved Solids of the Bio-treated Beverages Industry Wastewater with Penicillium Sp. after the 7th and 14th day of treatment (BIWW = Raw Beverages Industry Wastewater).

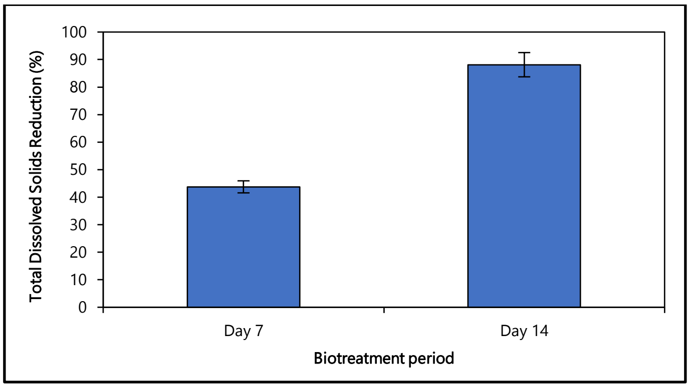

Figure 6. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

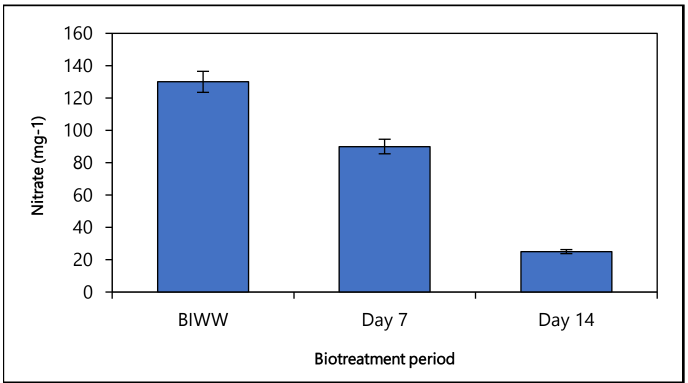

Figure 7. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

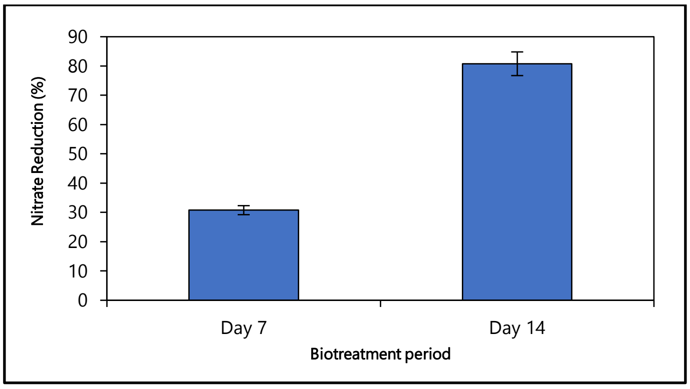

Figure 8. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

3.2. Total Dissolved Solid (TDS) and Nitrate

The Total Dissolved Solid (TDS) recorded for raw beverage wastewater is 800 mg/L (Figure 5). The TDS values obtained were generally less than 1000 mg/l, the upper limit set by WHO [39]. The value was later reduced to 450 and 95 mg/L with removal efficiencies of 43.75 and 88.13%, respectively, after 7 and 14 days of treatment with Penicillium Sp. (Figure 6). Chemical Oxygen Demand (COD) is the amount of oxygen consumed by the chemical breakdown of organic and inorganic matter.

The results obtained in this study showed a significant reduction of nitrate in raw beverage wastewater after bio-treatment with Penicillium Sp. for 14 days, with 90 and 25 mg/L at day 7 and 14, respectively, against 130 mg/L recorded for raw beverage wastewater (Figure 7). The higher percentage reduction efficiencies were recorded after 7 and 14 days of bio-treatment (30.77 and 80.77%), respectively (Figure 8).

3.3. Phosphate and Magnesium

A relatively higher rate of phosphate decrease (25 and 5 mg/L, Figure 9) with reduction efficiencies of (47.92 and 89.58%) (Figure 10 at days 7 and 14, respectively) was recorded in phosphate concentration after bio-treatment than the value observed for raw beverage wastewater at 48 mg/L. High phosphate levels will result in the eutrophication of the river.

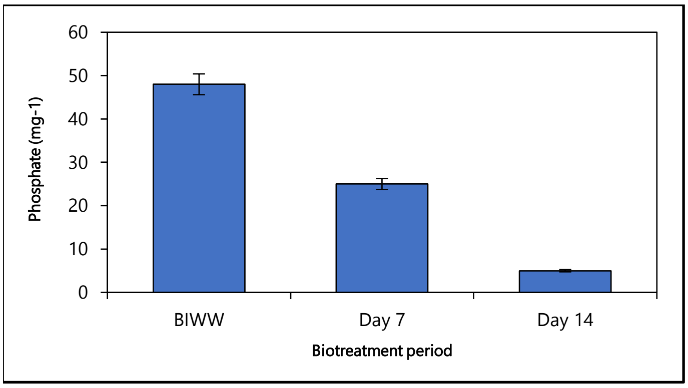

Figure 9. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

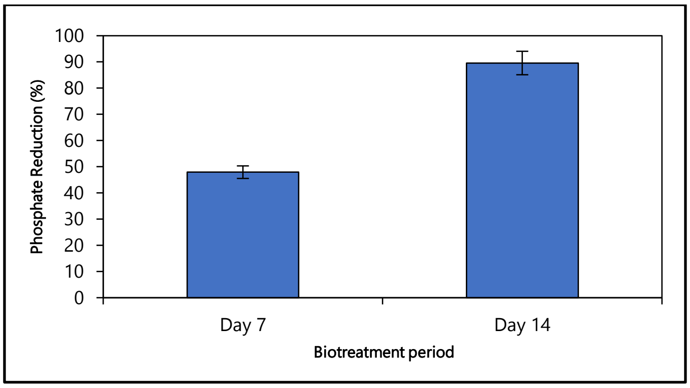

Figure 10. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

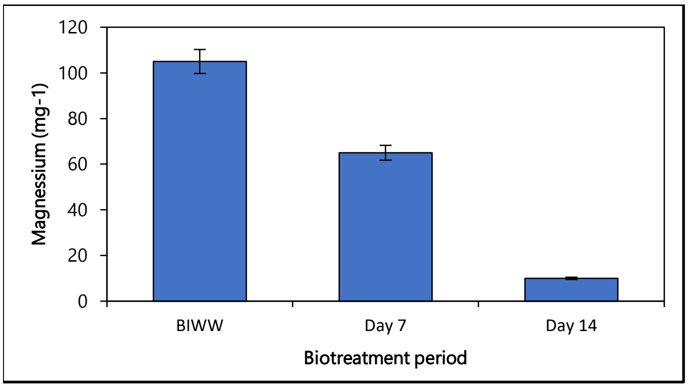

Figure 11. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

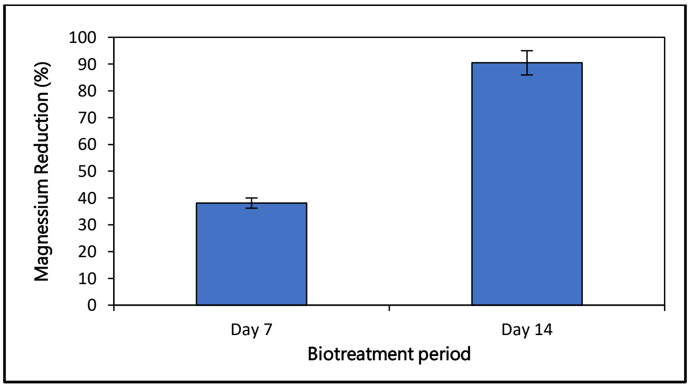

Figure 12. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

The results obtained in this study showed a significant reduction of magnesium in raw beverage wastewater after bio-treatment with Penicillium Sp. for 14 days, with 65 and 10 mg/L at day 7 and 14, respectively, against 105 mg/L recorded for raw beverage wastewater (Figure 11). The higher percentage reduction efficiencies were recorded after 7 and 14 days of bio-treatment (38.10 and 90.48%), respectively (Figure 12).

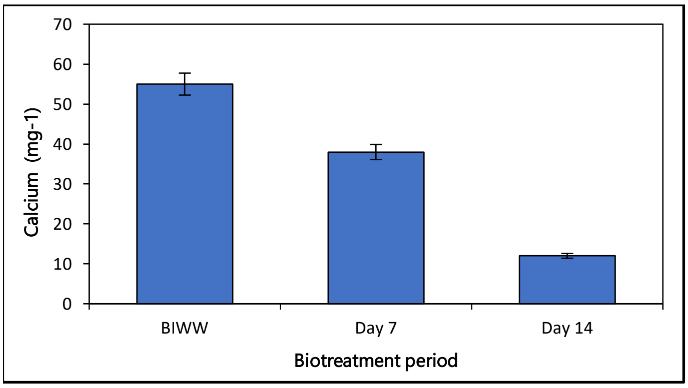

Figure 13. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

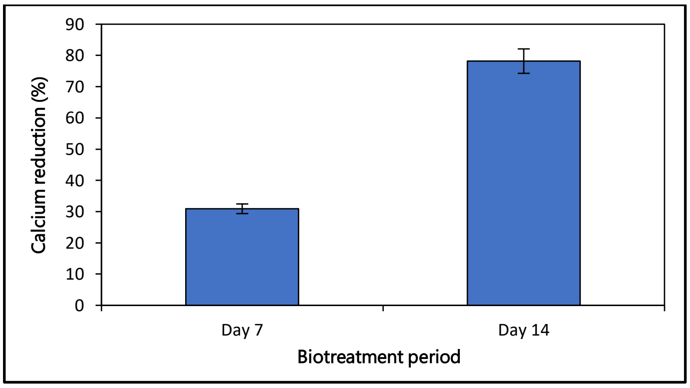

Figure 14. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

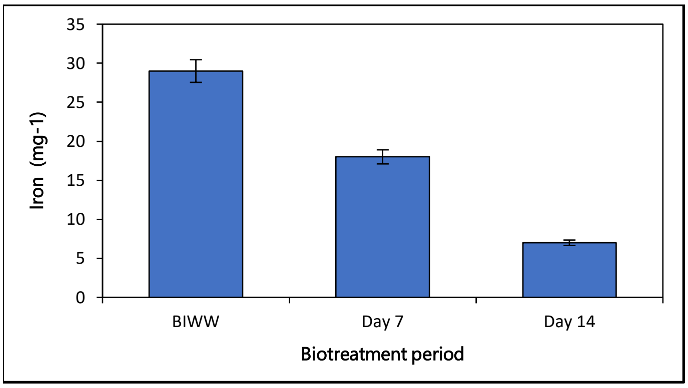

Figure 15. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

3.4. Calcium and Iron

The results of this study revealed a significant reduction of calcium in raw beverage wastewater after bio-treatment with Penicillium Sp. for 14 days, with 38 and 12 mg/L at day 7 and 14, respectively, against 55 mg/L recorded for raw beverage wastewater (Figure 13). The higher percentage reduction efficiencies were recorded after 7 and 14 days of bio-treatment (30.91 and 78.18%), respectively (Figure 14). The results obtained in this study showed a significant reduction of iron concentration in raw beverage wastewater after bio-treatment with Penicillium Sp. for 14 days, with 18 and 7 mg/L at day seven and as against 29 mg/L recorded for raw beverage wastewater (Figure 15). Higher percentage reduction efficiencies were recorded after 7 and 14 days of bio-treatment (37.93 and 75.86%), respectively (Figure 16).

3.5. Copper

Higher rate of copper decrease (0.07 and 0.02 mg/L Figure 9a) with reduction efficiencies of (22.22 and 77.78 % Figure 9b at day 7 and 14 respectively) was recorded in copper concentration after bio-treatment against value observed for raw local dye waste water 0.09 mg/L.

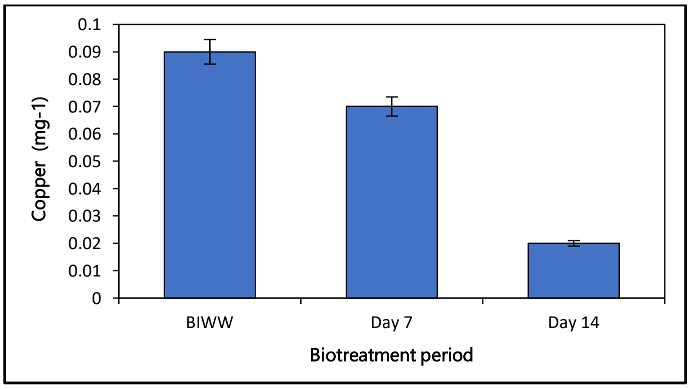

Figure 17. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

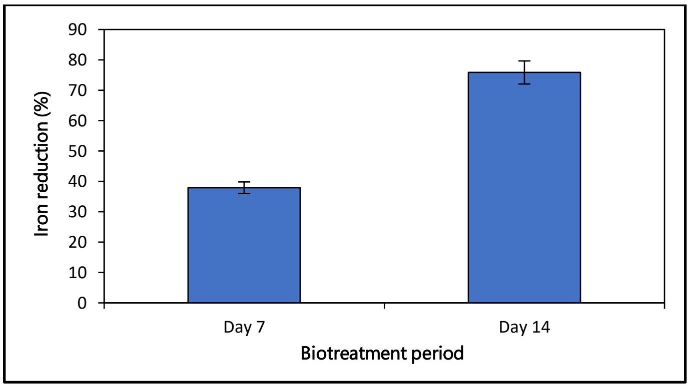

Figure 16. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

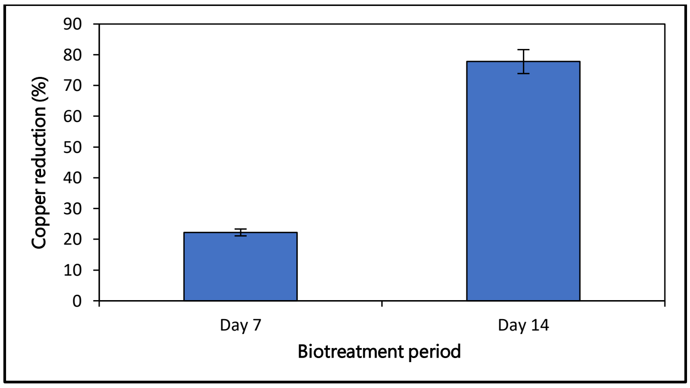

Figure 18. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

3.6. Zinc

Relatively higher rate of Zinc decrease (0.06 and 0.03±0.00 mg/L Figure 10a) with reduction efficiencies of (25 and 62.5 % Figure 10 b at day 7 and 14 respectively) was recorded in Zinc concentration after bio-treatment against value observed for raw beverages waste water 0.08 mg/L.

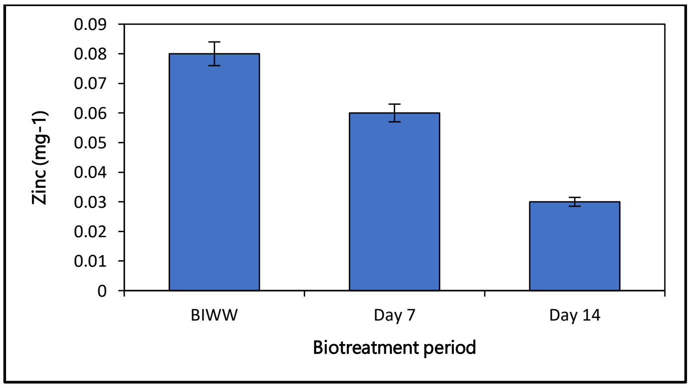

Figure 19. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

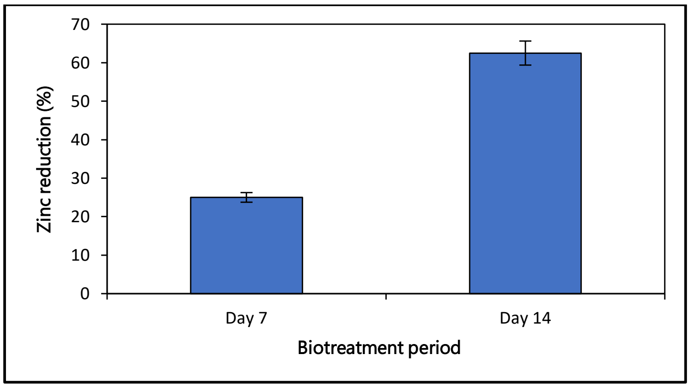

Figure 20. Total Dissolved Solids Removal efficiencies of Bio treated Pharmaceutical Wastewater after 7 and 14 days.

The results of physicochemical parameters and heavy metal content in beverage effluent samples were higher in untreated than treated. These results indicate that treatment methods employed by some companies in treating their effluents do not remove the parameters and heavy metals to WHO, USEPA, NESREA, and FEPA permissible levels before discharging the effluent into the river, and these could have serious environmental and health effects.

Our results showed that fungi (Penicillium sp.) were effective in removing raw beverage wastewater and it implies that it could simultaneously promote good algal growth. Fungi (Penicillium sp.) exhibited appreciable removal capacities of nutrients (ammonium-nitrogen, nitrate-nitrogen, phosphorus), BOD, and COD. Therefore, the treatment approach using fungi (Penicillium sp.) offers a low-cost, efficient, and environmentally friendly technology for treating mixed local dye-industrial wastewater.

4. Conclusion

Treatment of beverage wastewater, on the other hand, is a significant challenge because there is no specific and economically viable technique for adequately treating such a problem. Many traditional and emerging treatment approaches for beverage wastewater have been reported. Dye removal and degradation from dye-containing wastewater appear to be effective using physical. The microbial approach to beverage wastewater remediation is more cost-effective, environmentally friendly, and globally acceptable than physical and chemical methods.

However, one of the drawbacks of biological approaches is that they are less effective and should be administered over a long period. This work's recommendations are as follows:

- Adequate and prompt beverage industry wastewater treatment is crucial before discharge to the environment.
- The biological method of waste removal from beverage industry wastewater is effective.
- Those who practice fish farming should use microbial fungi (Penicillium sp.) to remove wastewater.
- The practice of using chemical methods of treating wastewater should be stopped.
- As a result, more research is required until an advanced, zero-waste process is established to minimize environmental and public health hazards during the transition from laboratory to pilot scale.

Acknowledgments

This research can be completed with the support by:

- Osun State College of Technology, Esa-Oke, Osun State, Nigeria;
- Raw Materials Research and Development Council (RMRDC) Abuja, Nigeria; and
- Obafemi Awolowo University, Ile Ife, Osun State, Nigeria.

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