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Comparative study of biodegradation potential of foreign and indigenous bacteria of pharmaceutical effluent

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Key Message: The study demonstrates how various bacterial strains can reduce pollutants like chemical oxygen demand (COD), total suspended solids (TSS), and total dissolved solids (TDS) in pharmaceutical wastewater. The study emphasizes how well microbial bioremediation works as an economical and environmentally beneficial substitute for chemical wastewater treatment.

Abstract

Pharmaceutical effluents, which contain organic compounds and other contaminants, are commonly found in wastewater. The ability of microorganisms to break down particular contaminants and their diversity are key components of this wastewater treatment procedure. A growing problem for the environment and human health is the constant release of these substances into surface waters. This study aimed to evaluate the biodegradation potential of indigenous and foreign bacteria in breaking down pharmaceutical effluents in the Irewolede region of Ilorin City, Kwara State, Nigeria, could break down the effluents. To ascertain the effectiveness of the treatment, the physicochemical characteristics of the pharmaceutical effluent were evaluated both before and after the procedure. Samples were collected from the effluent, and the biodegradation process was monitored using standard microbiological and analytical techniques.

Physicochemical properties including total dissolved solids (TDS), biochemical oxygen demand (BOD), total suspended solids (TSS), growth (Optical density, OD), pH, and degradation efficiency, were analyzed at the Department of Industrial Chemistry, University of Ilorin. Our investigation revealed that sample A had a high chemical oxygen demand (COD) value of 80.36 followed by sample B, indicating a higher level of organic pollution in sample A, followed by sample B. *Pseudomonas putida* showed less turbidity in effluent B, while seawater-isolated bacteria produced higher turbidity in sample A and moderate turbidity in sample B. While all bacterial isolates exhibited minimal reductions in BOD, they were effective in reducing TSS and TDS by over 50 %. This suggests that, while BOD reduction was limited, the bacteria could still contribute to the removal of suspended solids and dissolved solids from the effluent. This study demonstrated that *Bacillus* species, *E. coli*, *K. oxytoca*, *P. putida*, *Serratia marcescens*, and *Staphylococcus aureus* are promising bioremediation microbes for purifying pharmaceutical effluents and other organic wastes released into the environment. © 2024 The Author(s)

Keywords: Bacteria, Biodegradation, Bioremediation, Effluent, Environmental pollution, Microorganisms, Pharmaceutical industries

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Introduction

Industrial development is known as a way for economic development via new technologies. The manufacturing procedure of consumable products reveals the level of development of such a country especially Nigeria and other developing countries (Alam et al., 2024). Although, in every industry, effluents are obtained after industrial processes, the main problem is that the wastes produced are not monitored or treated by various methods like precipitation, filtration, and sedimentation among other treatments (Kato & Kansha, 2024). The release of effluent products into the environment causes various setbacks to the ecosystem. The local farmers practice cultivation close to the contaminated river due to effluents released from the

industry, channeling the water from such river for irrigation activity thereby causing harm to farm produce and toxic to the health of mankind (Nazir et al., 2024). This leads to the accumulation of heavy metals in the water bodies, soil, plants, animals, and environment.

In developing countries, industrialized effluents are partially treated or not treated, before it is released into the environment as the result of no knowledge or less manpower in the treatment of the waste (Singh et al., 2023). In Ilorin Nigeria, the Asa River is a prominent river that runs through an industrial area and the city at large. The discharge of waste produced into the river has resulted in the contamination of the river water. Jahan and Singh (2023) reported that in most cases industrial effluents from industrial companies have polluted dams and have

negative impacts on the plants and populace thereby causing pollution to the environment and alteration of the physico-chemical properties of the river. The accumulation of effluents in rivers suffocates organisms (fishes, prawns, etc) inhabiting the water bodies. However, some microbes present in the water can help in the neutralization of the toxicity that hinders the oxygen concentration, thereby promoting the suffocating rate of the organisms in the river (Ahmad et al., 2024). Due to their large wastewater discharge, pharmaceutical companies are of concern, since water is a crucial ingredient in the production of their product. Hence, huge amounts of wastewater are being discharged by these industries to the nearest river without being treated. However, this wastewater or effluent may not have passed through adequate treatment or not been treated at all, thereby causing water pollution to the receiving water body and the neighboring environment. During the process of manufacturing drugs in pharmaceutical industries, pharmaceutical effluents regarded as wastes are generated. The pathways involved in the production of drugs include extraction, processing, purification, and packaging thereby generating air emissions, liquid wastes, and solid wastes (Getino et al., 2024). Thousands of active substances are included in pharmaceuticals with biological and physicochemical features, which are not the same (Chia et al., 2024). Pharmaceutical products are needed in everyday activities as a result of their aid to improve our way of living, and they contribute to our health and living level.

According to Adeoye et al. (2024), pharmaceutical effluents' main sources of occurrence into the environment are the discharge of waste effluents from manufacturing industries and sewage treatment plants, the inappropriate disposal of unused or expired drugs, and accidental spills during production or distribution. High concentrations of organic compounds and total solids like mercury, cadmium, isomers of hexachlorocyclohexane, 1,2-dichloroethane, and solvents are mostly contained in pharmaceutical effluents. Various pharmaceutical effluents are however not consistent, among which is biochemical oxygen demand (BOD) is a measure of the amount of dissolved oxygen required by aerobic microorganisms to break down organic material in water over a specific period of time, usually five days at 20 °C, chemical oxygen demand (COD) which is a measure of the total amount of oxygen required to chemically oxidize both organic and inorganic compounds in water samples, suspended solids as well as phenol and pH, depending on the product manufactured, materials used, and the processing details (Adedayo et al., 2023b).

Biodegradation is the process by which organic pollutants are transformed into industrial effluents by the action of bacteria growth during metabolic processes that help maintain ecosystem and human health (Adedayo, 2024). Serious attention has been drawn due to the increase in contaminants from industrial activity and their hazardous effect on the environment and humans. Pharmaceutical product's biodegradation is affected by physical and chemical conditions (moisture content, temperature, pH, etc.), the nutritional requirements, stability of the active components as reported by Swaminaathan et al. (2024). The biodegradation of various

industrial wastes causing pollution is controlled by microorganisms has been reported by Saha and Rao (2024), these employed microbes utilized depending on the nature and source of the wastewater. Degradation ability of microorganisms such as bacterial species: *Bacillus amyloliquefaciens*, *B. mycoides*, *B. pumilus*, *B. thuringiensis*, *Arthobacter* spp, *A. viscosus*, *Micrococcus luteus*, *M. lylae*, *Acinetobacter baumannii* (Adedayo et al., 2023a; Vaishnav et al., 2024) and fungal species like *Aspergillus versicolor*, *A. flavus*, *Mucor circinellodis* etc (Dinakarkumar et al., 2024). This study aimed at a procedure involving microbial biodegradation of pharmaceutical effluents obtained at a flowing river in Kwara State, Nigeria. We hypothesize the bacteria isolated would biodegrade the effluents released into the river by the neighboring pharmaceutical company. These microbes reported are ecologically safe for humans, aquatic lives, and the environment compared to chemical utilization for degradation purposes.

Materials and Methods

Sample collection

Samples of pharmaceutical effluent were collected from the discharge point pass into a river at Irewolede area, Ilorin, Kwara State, Nigeria. They were collected in a clean sterile 5 liters container; the physicochemical properties were taken immediately after collection of the sample and were transferred immediately after collection to the laboratory.

Isolation of bacteria

At several locations of the industrial outflow line, wastewater samples were collected. Employing the pour plate method, the microbes were isolated on the nutrient agar (Gilchrist et al., 1973). The total colony count of bacteria was determined after the effluent was serially diluted, and the plate was inoculated in triplicate using 0.2 ml of the suitable dilution according to the method of Liu et al. (2008). The total colony count was calculated after the plates were incubated for 24 hours at 28 ± 2 °C. Serial dilution of the effluent was done in 6 folds (10^{-6}), aliquot was taken from 10^{-4} and 10^{-6} dilution and was then used for the isolation of microorganisms by pour plate method with nutrient agar. The microbial load of the effluent was then calculated, after 18 hrs incubation at 37 °C, both the microbial load and isolation of bacteria were done aseptically. The isolated bacteria were stored at 4 °C in a refrigerator for further analysis.

Characterization of bacteria isolates

Bacteria isolated from the effluent were characterized using morphological characteristics and biochemical tests according to Emerson and Moyer (1997).

Biochemical identification

Gram staining techniques

On glass slides, a thin smear was made from one-day-old pure cultures, the smeared slide was heated by passing over the flame. Two drops of KMnO_4 solution were added to the heat-fixed slides for a minute, after which the slide was rinsed under running distilled water. Furthermore, the washed slide was rinsed with Iodine solution for a minute, excess iodine solution was washed off with distilled water, 70 % alcohol as a decolorizer, and rinsed again with distilled water. The counter-staining procedure was adopted following the addition of 2 drops of Safranin for a minute and eventually washed with water, the slides were then air dried. The slide was observed under an oil immersion objective lens microscope for gram-negative and gram-positive bacteria (Bartholomew & Mittwer, 1952).

Spore staining technique

To the portion of heat-fixed slides containing a smear of organisms, 5 % Malachite green solution was added and steamed for a minute. The malachite green solution was then washed off with distilled water and decolorized for half a minute with safranin (two drops). The stained slides were air-dried and observed under an oil immersion objective lens (Beveridge et al., 2007).

Physicochemical properties of the effluent

This was carried out before and after the treatment to obtain the accuracy of the treatment. The method of Federation and Association (2005) was followed, and the collected samples were analyzed using standard methods to monitor the biodegradation process. Various properties (TDS, BOD, TSS, Growth (OD), pH, and efficiency of degradation) were analyzed.

Total Suspended Solids

Total suspended solids (TSS) were determined with a membrane filter apparatus following Viadero and Noblet (2002). About 100 ml of water sample was filtered through dried pre-weighed 0.45 μm filter paper and oven-dried at $105 \pm 5^\circ\text{C}$ for one hour. After this, the paper was cooled in a desiccator and weighed.

Biochemical oxygen demand

The water samples were properly shaken and 250 ml of each sample was taken aseptically into a 250 ml black bottle. The bottle was kept in the incubator at 20°C for 5 days. After 5 days of incubation, the dissolved oxygen analyzer; Model JPB-607 was used to determine the final dissolved oxygen. The analyzer was calibrated in distilled water before and after use for each sample. The experiment was carried out in triplicates to enable proper statistical evaluation following the method of Cude (2001).

Total dissolved solids and dissolved oxygen

The total dissolved solid and dissolved oxygen of the effluent was carried out according to Ebeling et al. (2006).

Screening for organic degrading activity of the isolates from pharmaceutical effluent

Bacterial isolates were screened for their ability to degrade the organic contents by determining the degree of turbidity of pharmaceutical effluent based on their average growth rate, on minimal salt medium (g/l) (KH_2PO_4 0.05, $(\text{NH}_4)_2\text{SO}_4$ 0.05, $\text{MgSO}_4 \cdot 2\text{H}_2\text{O}$ 0.05, agar 15 amended with 1% (v/v)). The filtered sterile pharmaceutical effluent acts as the sole carbon source. The same composition of salt except 1.0 v/v filtered sterile industrial effluent was used as the control plate. All the experiments were carried out in duplicates. The bacteria within the effluent were observed for their biodegradability potential on effluent against those that are isolated from seawater. The ability of the bacterial isolates to utilize the industrial effluent as the sole source of carbon and energy was determined using the method of Yoshimoto et al. (2004) with little modification for vegetable oil wastewater degradation studies.

Biodegradation experiment

The extent of biodegradation activity among isolates was conducted in 250 ml conical flasks containing 100 ml of each effluent and incubated for 144 h at 30°C respectively. The biodegradation ability of both natural and organisms isolated from seawater was observed using the rate of reduction in the following physicochemical OD_{540} nm, BOD_5 , COD, TDS, and TSS. Each organism was tested on each of the two effluents. The experiment was done according to Yoshimoto et al. (2004) with little modification.

Results

The bacterial population in the effluent is $2.15 \cdot 10^5$ c.f.u./ml. A dense population of bacteria in the pharmaceutical effluents is indicated by the high microbial load index (10^5). Additionally, according to the coliform index, it contained coliform *Bacillus* species and *E. coli* with a high MPN (1800). The contamination of the effluent is indicated by the isolation of *E. coli* and *Bacillus* species in every tube used for the coliform test. Strains of Ty5 (*Klebsiella oxytoca*), Pp2 (*Bacillus cereus*), Ty3 (*Pseudomonas aeruginosa*), Ty4 (*Pseudomonas* species), and Ty9 (*Pseudomonas putida*) were isolated from effluent obtained from seawater (Table 1) and Mg2 (*Staphylococcus aureus*), Mg1 (*Escherichia coli*), Mg5 (*Serratia marcescens*), Mg9 (*Salmonella* spp), and Mg11 (*Enterococcus* species), were isolated from the pharmaceutical wastewater (Table 1). All these microbes have been reported to cause disease in humans (Sammal et al., 2024), livestock (Schwab et al., 2024), and aquatic life (Chen et al., 2024), and cause pollution in the environment they were isolated from (Evoung Chandja et al., 2024).

Table 1 Screening for a bacterial isolate that utilizes effluent collected as the sole carbon source in seawater and pharmaceutical effluents

Isolates	Effluent A	Effluent B
Ty4 (<i>Pseudomonas species</i>)	++	++
Ty5 (<i>Klebsiella oxytoca</i>)	+++	++
Mg2 (<i>Staphylococcus aureus</i>)	+++	++
Mg1 (<i>Escherichia coli</i>)	+++	++
Mg5 (<i>Serratia marcescens</i>)	+++	++
Mg9 (<i>Salmonella spp</i>)	+++	++
Mg11 (<i>Enterococcus species</i>)	++	++
Ty9 (<i>P. putida</i>)	+	++
Ty3 (<i>P. aeruginosa</i>)	+++	++
Pp2 (<i>B. cereus</i>)	+++	++

+ = Less turbid, ++ = Turbid, +++ = Highly turbid

Physiochemical properties of samples

The physiochemical properties of the effluents of samples A and B are presented in Table 2. These results show that effluent sample A has high COD which can be related to the vast varieties of chemicals used in the manufacturing of their product, which is also present in the effluent, effluent sample A also has low dissolved oxygen available, effluent sample B has high total dissolved solids and total suspended solids, effluent sample B has high pH indicating high alkaline nature of the effluent. However, Effluent sample B shows a lesser microbial load compared to the load on effluent sample A, attributed to the highly alkaline nature of the effluent.

Samples A and B displayed their turbidity and the effectiveness of the microbial isolates in each sample which contributed to the turbidity nature of the samples. *Klebsiella oxytoca*, Mg2 (*Staphylococcus aureus*), Mg1 (*Escherichia coli*), Mg5 (*Serratia marcescens*), *Bacillus cereus*, *Pseudomonas aeruginosa* and Mg7 (*Salmonella spp*) appear to contribute to high turbidity of the effluent A while *Pseudomonas species* and Mg11 (*Enterococcus species*) are turbid and *Pseudomonas putida* is less turbid in effluent A. Moreover, in effluent B, high turbidity was not recorded. However, turbid was obtained as a result of the following isolates *Klebsiella oxytoca*, Mg2 (*Staphylococcus aureus*), Mg1 (*Escherichia coli*), Mg5 (*Serratia marcescens*), *Bacillus cereus*, *Pseudomonas aeruginosa*, Mg9 (*Salmonella spp*), *Pseudomonas species* and Mg11 (*Enterococcus species*), and *Pseudomonas putida* but less turbid was not obtained in effluent B. Bacteria isolated from seawater are known to be highly

turbid in effluent sample A, with moderate turbid in effluent sample B, *Pseudomonas putida* showing less turbid in effluent B.

Effluent samples including high levels of chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), total dissolved solids (TDS), heavy metals, and hazardous organic compounds are probably present in the employed Effluent samples. These contaminants can be decreased by the biodegradation and bioremediation abilities of organisms isolated from saltwater and effluent samples. Table 3 – 6 shows the potential of the isolated microbes to biodegrade the samples. The isolates (Mg5, Mg1, Mg11, and Mg9) displayed the highest percentage of reduction levels in the effluent sample obtained from seawater, while TDS showed the highest percentage of reduction in Mg2 as observed in Table 3. Table 3 shows the potential of organisms isolated from seawater on effluent samples A, where Mg5, and Mg11 have the highest COD reduction with more than 80% reduction, and the rest showing more than 50% reduction thus have a high COD reduction, all isolates show low BOD reduction. Mg2 and Mg11 show a moderate reduction in TSS and TDS with more than 50% reduction, all isolated have low reduction in TDS.

In the effluent sample, Table 4 there is a high percentage of reduction in TDS for the following isolates Ty4, Ty5, Ty3, and Pp2 while COD has a high reduction percentage for Ty9. Table 4 shows the action of indigenous organisms on effluent sample A. *Pseudomonas putida* has a high COD reduction rate among all isolates, followed by *Pseudomonas spp*, *Klebsiella oxytoca* and *Bacillus cereus* and *Pseudomonas aeruginosa* respectively. All isolates show low BOD reduction. All isolates show more than a 50% reduction in TSS, and TDS. According to the details in Table 5, Mg2, Mg1, and Mg9, displayed a high reduction percentage in BOD, while Mg5 and Mg11 displayed a high percentage of reduction in COD. However, Table 6 revealed that Ty4, Ty3, and Pp2 have a high percentage reduction in BOD while Ty5 and Ty3 have a high percentage reduction in COD. Table 5 shows the action of the organism isolated from seawater on the effluent sample, Mg11 has a high COD reduction rate with most isolates having more than 50 % except Mg 1 having a low reduction in COD. All isolates show a high BOD reduction of more than 50 %. All isolates have a low reduction rate in TDS and TSS. Table 6 shows the action of indigenous bacteria on effluent sample B., Pp2 has a high COD reduction with the rest having more than 50% reduction, all isolates also show high BOD reduction, and all isolates show low TSS and TDS.

Table 2 Initial physicochemical properties of industrial effluent

Properties	COD (mg/ml)	BOD (mg/ml)	DO (mg/ml)	Hardness (mg/ml)	TSS (mg/ml)	TDS (mg/ml)
Effluent sample A	80.36	14.1	10.32	55.08	688	1190
Effluent sample B	70.36	21.8	15.52	54.24	712	1290

COD = Chemical oxygen demand, BOD = Biological oxygen demand, DO = Optical density, TSS = Total suspended solids (mg/ml), TDS = Total dissolved solid

Table 2 continue

Properties	NO ₃ ⁻ (mg/ml)	PO ₄ ⁻ (mg/ml)	pH	Temperature (°C)	Microbial load (CFU/ml)
Effluent sample A	5.36	0.56	7.3	25	1.63 × 10 ⁴
Effluent sample B	5.02	0.63	10.7	25	1.0 × 10 ⁴

NO₃⁻ = Nitrate, PO₄⁻ = Phosphate

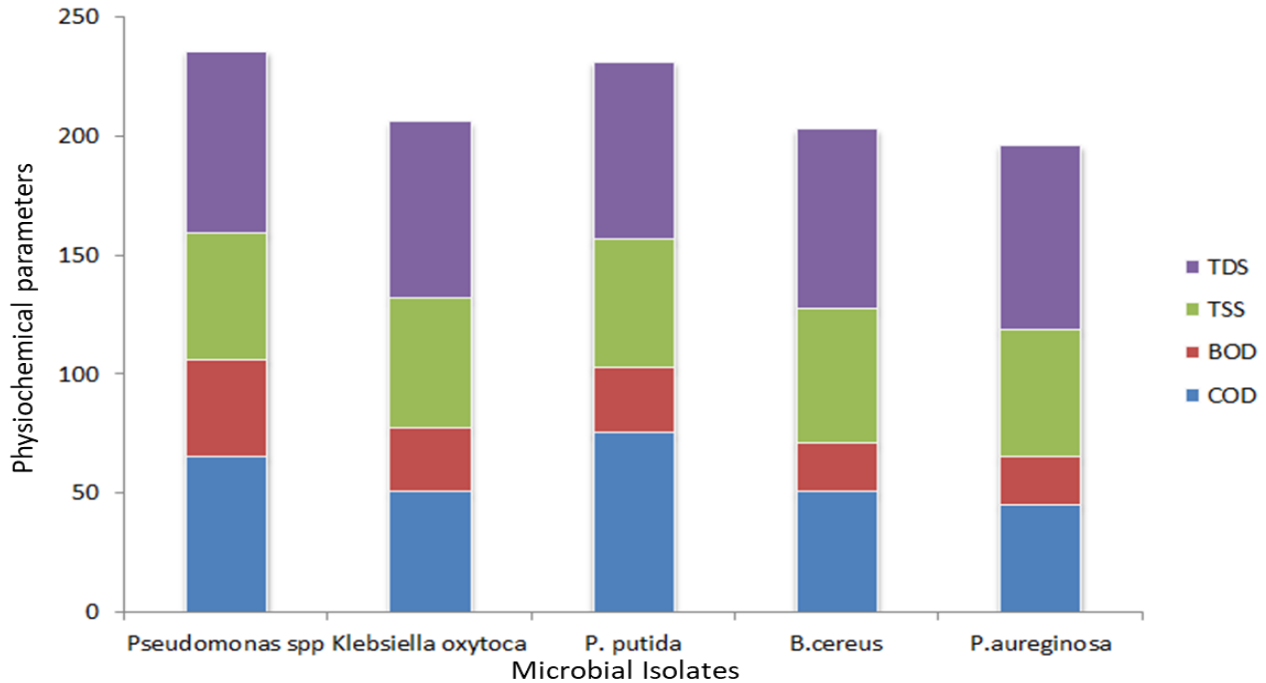


Fig. 1 Showing reduction in the physicochemical properties on effluent sample A by indigenous organism. The physicochemical properties displayed reveal how the effectiveness of each microbial isolate on the percentage reduction of the employed samples BOD, COD, TSS, and TDS

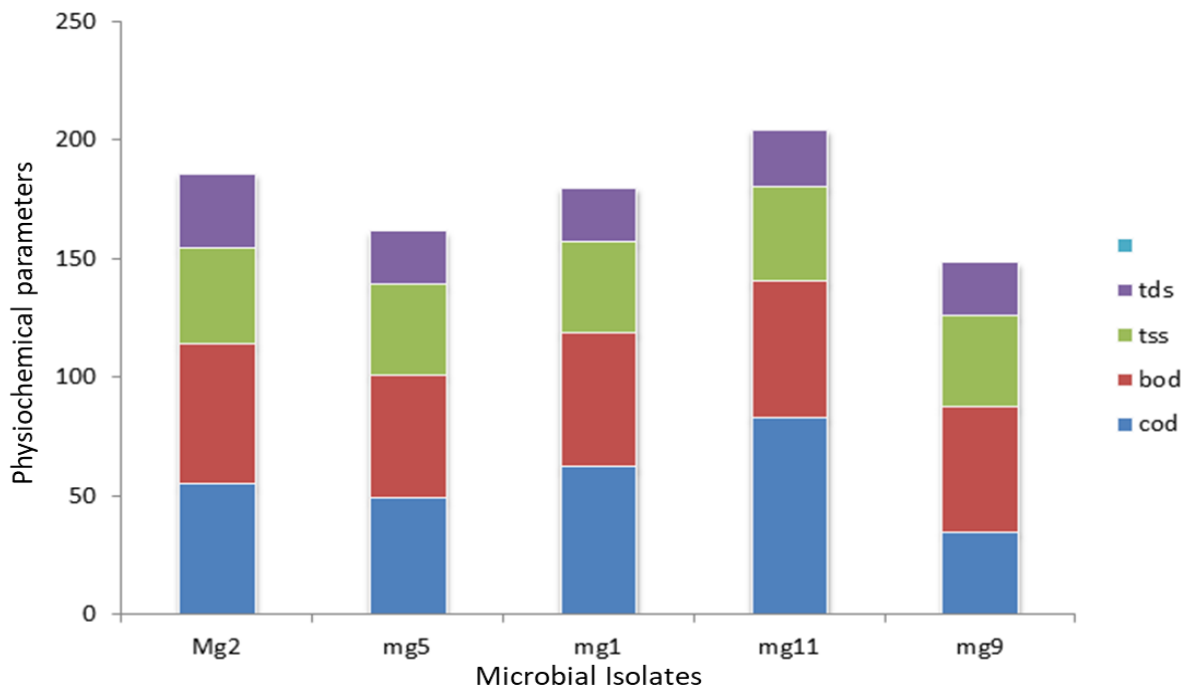


Fig. 2 Graphical representation showing reduction in physicochemical properties on effluent sample B by bacteria isolated from seawater. The physicochemical properties displayed reveal how the effectiveness of each microbial isolate on the percentage reduction of the employed samples BOD, COD, TSS, and TDS

Table 3 Reduction in physicochemical properties of effluent A using organism isolated from seawater

Properties	Control (mg/ml)	Mg2 (mg/ml)	Reduction (%)	Mg5 (mg/ml)	Reduction (%)	Mg1 (mg/ml)	Reduction (%)	Mg11 (mg/ml)	Reduction (%)	Mg9 (mg/ml)	Reduction (%)
COD	80.8	28.0	65.3	14.0	82.6	28.0	65.6	14.8	81.7	46.0	43.0
BOD	14.1	11.2	20.6	9.2	34.75	11.1	21.9	10.1	28.4	10.3	27.0
TSS	688	301	56.3	420	38.1	329	28.4	330	52.0	444	35.5
TDS	1190	298	74.9	860	27.7	370	27.0	373	68.6	997	16.2

COD = Chemical oxygen demand, BOD = Biological oxygen demand, TSS = Total suspended solids (mg/ml), TDS = Total dissolved solid, Mg1 = *Escherichi coli*, Mg2 = *Staphylococcus aureus*, Mg5 = *Serratia marcescens*, Mg9 = *Salmonella* spp, Mg11 = *Enterococcus* species

Table 4 Reduction in the physicochemical properties of effluent sample A by indigenous organism

Properties	Control (mg/ml)	Ty4 (mg/ml)	Reduction (%)	Ty5 (mg/ml)	Reduction (%)	Ty9 (mg/ml)	Reduction (%)	Ty3 (mg/ml)	Reduction (%)	Pp2 (mg/ml)	Reduction (%)
COD	80.8	28.0	65.4	40.0	50.4	20.0	75.5	40.0	50.4	44.0	45.0
BOD	14.1	8.4	40.4	10.3	27.0	10.2	27.7	11.20	20.6	11.3	19.9
TSS	688	322	53.2	315	54.2	320	53.5	300	56.4	320	53.5
TDS	1190	303	76.3	308	74.1	303	73.5	296	75.1	268	77.5

COD = Chemical oxygen demand, BOD = Biological oxygen demand, TSS = Total suspended solids (mg/ml), TDS = Total dissolved solid, Ty3 = *P. aeruginosa*, Ty4 = *Psuedomonas* species, Ty5 = *Klebsiella oxytoca*, Ty9 = *P. putida*, Pp2 = *Bacillus cereus*

Table 5 Reduction in physicochemical properties on effluent sample B by bacteria isolated from seawater

Properties	Control (mg/ml)	Mg2 (mg/ml)	Reduction (%)	Mg5 (mg/ml)	Reduction (%)	Mg1 (mg/ml)	Reduction (%)	Mg11 (mg/ml)	Reduction (%)	Mg9 (mg/ml)	Reduction (%)
COD	70.3	32.0	54.2	29.4	58.8	66.0	6.2	12.0	82.9	46.0	34.6
BOD	21.8	8.9	59.2	10.5	51.8	9.4	56.9	9.3	57.3	10.3	52.7
TSS	712	425	40.3	441.0	38.2	440	38.2	430	39.6	444	38.2
TDS	1290	882	31.6	998	22.6	997	22.7	976	24.3	997	22.7

COD = Chemical oxygen demand, BOD = Biological oxygen demand, TSS = Total suspended solids (mg/ml), TDS = Total dissolved solid, Mg1 = *Escherichi coli*, Mg2 = *Staphylococcus aureus*, Mg5 = *Serratia marcescens*, Mg9 = *Salmonella* spp, Mg11 = *Enterococcus* species

Table 6 Reduction in the physicochemical properties of effluent sample B by indigenous organism

Properties	Control (mg/ml)	Ty4 (mg/ml)	Reduction (%)	Ty5 (mg/ml)	Reduction (%)	Ty9 (mg/ml)	Reduction (%)	Ty3 (mg/ml)	Reduction (%)	Pp2 (mg/ml)	Reduction (%)
COD	70.3	28.0	60.2	58.0	17.6	66.0	6.1	32.0	54.5	14.0	80.2
BOD	21.8	10.4	52.3	8.5	61.0	9.2	57.8	10.10	53.6	9.2	57.7
TSS	712	421	40.9	430	39.6	520	26.9	510	28.3	42.0	41.0
TDS	1290	864	33.0	976	24.3	1020	20.9	1010	21.7	860	33.3

COD = Chemical oxygen demand, BOD = Biological oxygen demand, TSS = Total suspended solids (mg/ml), TDS = Total dissolved solid, Ty3 = *P. aeruginosa*, Ty4 = *Psuedomonas* species, Ty5 = *Klebsiella oxytoca*, Ty9 = *P. putida*, Pp2 = *Bacillus cereus*

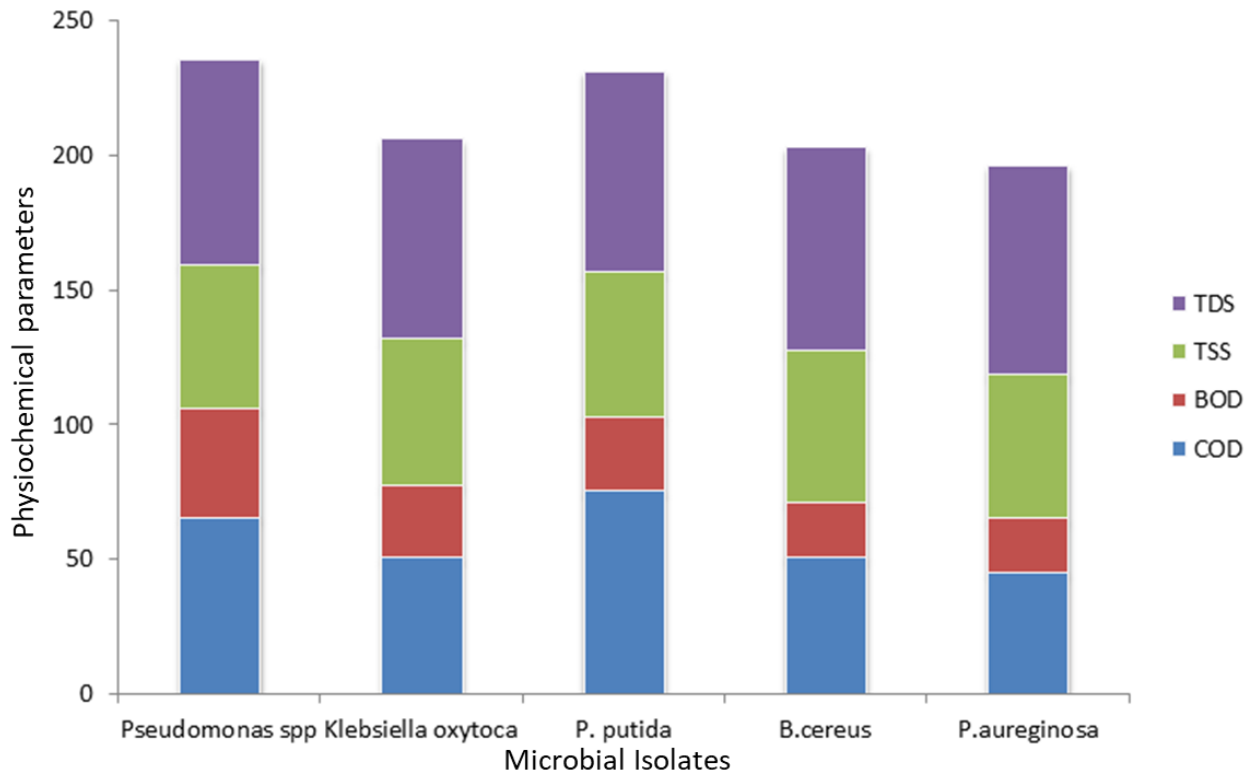


Fig. 3 Reduction in physicochemical properties of effluent sample B by indigenous microbes
The physicochemical properties displayed reveal how the effectiveness of each microbial isolate on the percentage reduction of the employed samples BOD, COD, TSS, and TDS

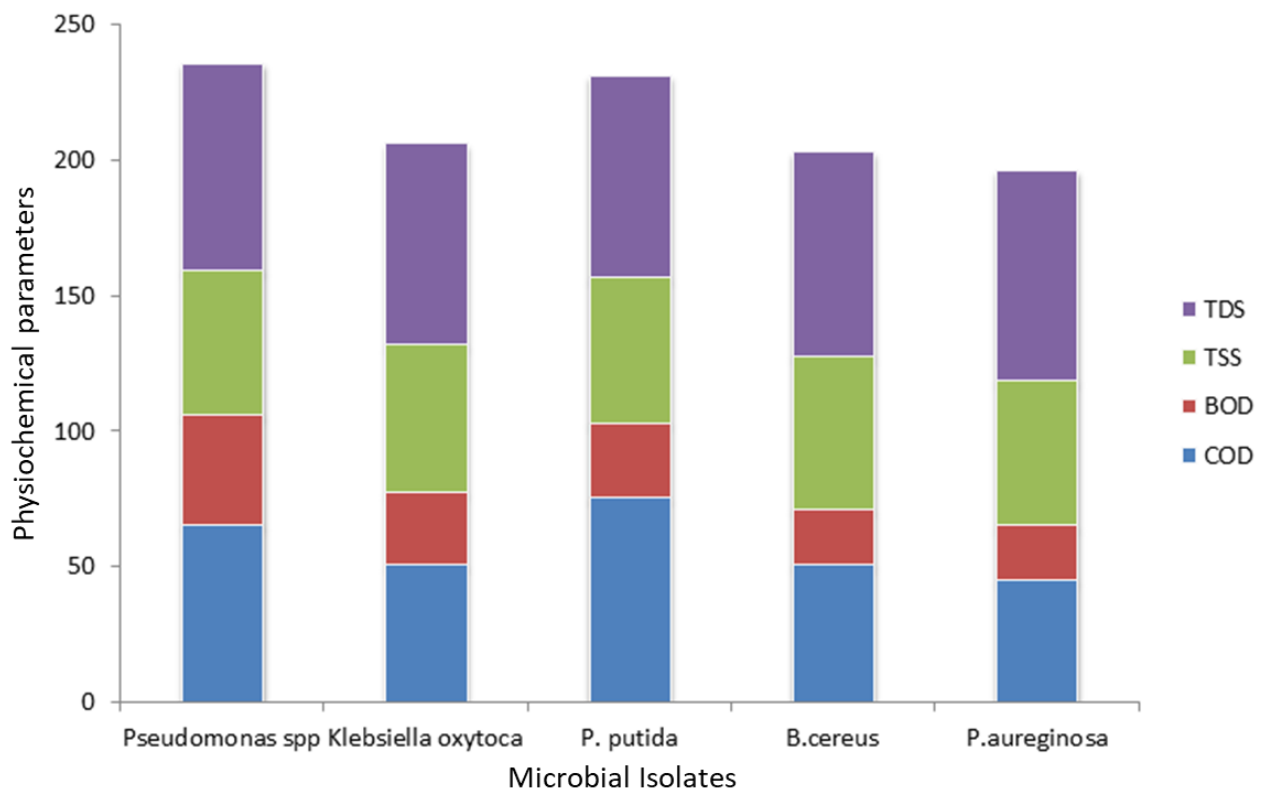


Fig. 4 Rate of reduction of physicochemical properties of effluent sample B by indigenous organism.
The physicochemical properties displayed reveal how the effectiveness of each microbial isolate on the percentage reduction of the employed samples BOD, COD, TSS, and TDS

Discussion

Effluents are untreated waste materials that can be liquid, semisolid, or solid waste produced from industry and directly or indirectly released to the environment. In this study, some bacteria species potential were observed in the biodegradation of effluent wastes. Numerous contaminants found in wastewater, especially from sectors like pharmaceuticals, alter its physicochemical properties. Total dissolved solids (TDS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), and pH are the most important factors used to assess effluent quality. Prior to being released into water bodies, these characteristics assess the wastewater's toxicity, environmental impact, and treatability. The microbial population reported in this study coincides with the results of this investigation and is comparable to those previously documented for microbial communities in several Nigerian rivers that are contaminated by industrial, agricultural, and human waste (Chukwura & Okpokwasili, 1997). They were utilized because of their beneficial role in treating the environmental challenges and not harmful to humans, animals, and the environment. Mun et al. (2024) reported how iron-reducing bacteria have been used to control the anaerobic biodegradation of chlorophenol in acclimated sludge which coincides with our hypothesis of this research. Other studies have reported more facts on the effluents released by manufacturing industries and how the waste was controlled (Govil et al., 2024; Wani & Mishra, 2024).

This study presents the result of the effluent sample obtained at the river in which effluent B reveals a lesser microbial load compared to the load on effluent sample A, attributed to the highly alkaline nature of the effluent. The isolated organisms are Ty5, Mg2, Mg1, Mg5, Mg11, Ty9, Ty3 and Pp2. How much they are present in the effluents A and B are displayed in Table 1. A facultative anaerobic, Gram-negative bacteria, *Escherichia coli* is frequently found in both human and animal digestive systems. Some strains have been investigated for their capacity to biodegrade organic and inorganic contaminants in industrial and pharmaceutical effluents (Yang et al., 2024), while others are pathogenic. *E. coli* has been studied for use in bioremediation because of its rapid growth, genetic plasticity, and metabolic variety. *Pseudomonas* species are rod-shaped, motile, gram-negative bacteria that are well-known for their capacity for biodegradation and metabolic flexibility. They are perfect candidates for bioremediation because they flourish in a variety of settings, such as pharmaceutical and industrial effluents. By breaking down organic contaminants, hydrocarbons, heavy metals, and medications, these bacteria can lessen environmental pollution (Sivasamy et al., 2024). *Bacillus* species are rod-shaped, spore-forming, Gram-positive bacteria whose capacity for biodegradation has been extensively researched. They are perfect candidates for wastewater and effluent treatment because of their capacity to withstand harsh conditions, generate hydrolytic enzymes, and metabolize a variety of contaminants. Organic materials, heavy metals, petroleum hydrocarbons, dyes, and pharmaceutical residues can all be effectively broken down by them (Akinsemolu et al., 2024). Gram-negative, rod-

shaped, facultative anaerobe *Klebsiella oxytoca* is a member of the Enterobacteriaceae family. It is frequently found in wastewater, soil, water, and animal digestive systems. Some strains have demonstrated considerable biodegradation capacity in treating industrial, pharmaceutical, and petroleum-based effluents, whereas other strains are opportunistic pathogens (Ayodele et al., 2024). A broad variety of organic contaminants, heavy metals, and nitrogenous substances may be broken down by the organism adaptable metabolic system in both aerobic and anaerobic settings. Other organisms that has been reported include *Serratia marcescens*, *Enterococcus* species, etc.

The pH scale, which ranges from 0 to 14, indicates how acidic or alkaline the effluent is. Acids, bases, or buffer solutions found in wastewater treatment effluents can change the pH of natural water, impact aquatic life, and alter microbial activity (Eryildiz-Yesir et al., 2024). Acids and bases from chemical processes (such as solvents and medication production) are the sources of pH alteration. Other sources include pharmaceuticals that employ buffering agents and organic molecules or heavy metals dissolving (Nyamba et al., 2024). The physicochemical analysis of the two effluents used in this research work displayed favorable pH in the effluents due to the nature of the raw materials used in the process of manufacturing the products (Fig. 1-4). According to Bohlok et al. (2024), the pH range accepted for edible foods and drinks ranges between 6.5 to 8.5. In this present study, the pH of effluent A is 7.3 was in line with the pH of the edible food and drink while effluent B has a pH of 10.7. Our finding coincides with the study presented by Rahman et al. (2024) which reported the pH scale of 6 – 9 in high-strength wastewater and reuse in arid and semi-arid regions.

Biochemical oxygen demand (BOD) indicates the amount of oxygen bacteria require to decompose organic materials in wastewater for five days at 20 °C (Lacalamita et al., 2024). A high BOD is a sign of organic pollution, which can cause aquatic mortality by reducing the amount of dissolved oxygen in water bodies. The BOD reduces the amount of oxygen in receiving water bodies, which leads to the suffocation of aquatic life and ecological imbalance, it encourages anaerobic conditions, which causes harmful gases (methane, H₂S) to accumulate, and decreases the biological wastewater treatment systems' efficiency (Janga et al., 2024). Our result revealed the BOD level ranging from 14 – 22 mg/ml in the samples and reported microbes reduced the level of BOD by less than 55 % in the effluent received from the pharmaceutical industry and the seawater. Our study result is in line with the report of Hassen et al. (2022) that reported the treatment processes used in Egyptian plants' chemical and microbiological tests conducted on wastewater released by a manufacturing industry along the Mediterranean Sea 50 % reduction.

Chemical oxygen demand (COD) on the other hand measures the amount of oxygen needed to chemically oxidize organic materials, both biodegradable and non-biodegradable. This was because COD takes into account the oxygen demand from synthetic, hazardous, and non-biodegradable materials, it is usually greater than BOD (Lacalamita et al., 2024). The COD shows the presence of

contaminants that need sophisticated treatment since they are not biodegradable. Elevated COD causes eutrophication and toxicity by lowering the amount of oxygen in aquatic bodies. Impacts the effectiveness of biological therapy (e.g., aerobic bacteria have difficulty breaking down certain pollutants). High COD is caused by synthetic chemicals, solvents, dyes, and residual medicinal substances (such as hormones, antibiotics, and analgesics), detergents, and surfactants used in the production of drugs (Devanathan et al., 2024). In this study, the COD level obtained ranged from 25 – 80 mg/ml. However, the percentage of COD reduction ranges from 50 – 85 % which was in line with the study conducted by Kapdan and Alparslan (2005) who employed anaerobic-aerobic sequential treatment on real textile wastewater and the result showed a 90 % reduction of COD. The COD permissible standard is less than 4.0 mg/ml according to the USPH standard, and the COD of both effluents collected was considerably higher than the permissible standard (Table 3-6).

The entire amount of dissolved organic and inorganic materials in wastewater is referred to as total dissolved solids (TDS). Aquatic life, soil permeability, and water quality are all impacted by high TDS which causes water to become salinized, rendering it unfit for drinking or irrigation. Industrial equipment is impacted by high salt concentration (corrosion, scaling) and may alter metabolism in aquatic species by causing osmotic stress. TDS content measures the salinity comprised of a large number of varieties of salt. Although, the standard required for TDS varies with water permissible range for drinking is up to 100 mg/ml, for irrigation is 200 mg/ml, and not useful for drinking and irrigation is > 300 mg/ml (Patra et al., 2024). As observed in Table 2, TDS values (688 and 712) obtained for effluent A and B were higher than the range permitted for both drinking and irrigation purposes. The result of biochemical characterization showed that of the four isolates from pharmaceutical effluent. Two belong to the genus *Pseudomonas* and the only isolates from beverage effluent also belong to the same genus. *Pseudomonas* has been reported to be recalcitrant to some toxic chemicals in pharmaceutical effluents that may be toxic to other bacteria, due to the presence of certain resistance mechanisms (Saeed et al., 2024).

Conclusion

Applied chemicals become uneconomical and cause further environmental damage, hence, economical and ecofriendly techniques using bacteria can be applied for fine-tuning wastewater. Biotreatment offers a cheap and effective alternative for the color removal of textile dye. Microbial evolution is rapid, and for this reason, this research work has contributed tremendously to new genetic pathways for breaking down many anthropogenic chemicals that have not existed for more than a few decades. In this sense, microbes are useful resources for cleaning up contaminated environments. Thus, by this study, it is concluded that bacterial isolates like *Bacillus* sp, *E. coli*, *K. oxytoca*, and *P. species*, etc. can be used as good microbial sources for wastewater treatment. Further studies on the identification of the bacterial isolates and the mechanism responsible for

the biodegradation of industrial effluents should be determined. More basic research into this potentially useful type of metabolism is required, as attempts to try to scale up techniques that have shown promise on a laboratory scale. Also, the Government should always check the activities of agencies saddled with the responsibility of monitoring environmental pollution to reduce the havoc caused by this effluent on the environment and aquatic inhabitants at large.

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