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RESEARCH ARTICLE

Impact of Effective Microorganisms on the Microbiological and Physicochemical Parameters of Produced Water

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ABSTRACT

The study investigated the impact of effective microorganisms on the microbiological and physicochemical parameters of produced water. Produced water was obtained from Ebocha oil field within Rivers state. The spread plate method was used to determine the total heterotrophic bacterial counts, and pure isolates were subjected to biochemical characterization. Pure bacterial suspension of *Lactobacillus plantarum, Aspergillus* sp. and *Penicillium italicum* were obtained and subsequently inoculated into wastewater sample in consortium. Treated (with the consortium) wastewater sample was analyzed at interval for their physicochemical and microbiological parameters during the appropriate period of incubation (14 days) at room temperature. The bacterial load of produced water decreased from 1.3 x 10^8 cfu/ml to 3.6 x 10^7 cfu/ml, coliform counts increased from 2.0 x 10^5 cfu/ml to 1.4 x 10^6 cfu/ml, Staphylococcal counts decreased from 3.4 x 10^5 cfu/ml to 0 cfu/ml and *Vibrio* counts decreased from 8.0 x 10^5 cfu/ml to 2.0 x 10^5 cfu/ml. Biochemical characterization of bacterial isolates from the wastewater revealed the presence of *Enterobacter* sp, *Bacillus* sp, *Klebsiella* sp, *Proteus* sp, *Escherichia coli* and *Staphylococcus* sp. The pH and temperature ranges of water sample during the period of treatment varied. The BOD values decreased from 240 mg/L to 21 mg/L while the COD values decreased from 400 mg/L to 160 mg/L. The results of the heavy metal assessment revealed an appreciable reduction in the heavy metal concentrations of the sample. The concentration of zinc in produced water decreased from 0.113 mg/L to an undetectable level (< 0.01 mg/L), iron concentration decreased from 1.071 mg/L to 0.139 mg/L, Nickel decreased from 2.110 mg/L to 1.081 mg/L while copper and cadmium were undetected. The use of effective microorganisms in the treatment of produced water was effective in the reduction of microbial load, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and removal of heavy metals.

Keywords: Effective microorganisms, Produced water, Heavy metals, Treatment

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1 Introduction

For decades, the rapid population growth and intense industrial activities have led to the disturbance and pollution of the environment inhabited and relied upon by humans and other animals [1]. Same can be said of the aquatic habitat which has been at the receiving end of untreated municipal and industrial effluent. These wastewaters, when initially discharged into the waterbodies are diffused through the water and pollutants can easily be degraded because of the low concentration. However, when the concentration of organics and inorganics becomes high on subsequent discharge, parameters such as biochemical oxygen demand (BOD) (ppm), chemical oxygen demand (COD) (ppm) and suspended solids (ppm) increases to tens of thousands. Some health risk associated contaminants such as heavy metals, pesticides, ammonia etc can also be introduced into the waterbodies through the discharge of effluents [2]. Groundwater and surface water systems in the urban areas have been affected by the indiscriminate disposal of waste. Effluents and sewage from both domestic and commercial sources are disposed directly into freshwater bodies in most developing countries [3]. Sometimes, people in the cities dispose their municipal waste directly into drainages which becomes stagnated and provides a breeding environment for mosquitoes, microorganisms and other pests [3]. Wastewater treatment is therefore an important factor in environmental protection. There are several conventional methods of wastewater treatment and this include the activated sludge which uses a combination of different physiological groups of microorganisms. These microorganisms break down organic substances in to simpler and environmental friendly substances rendering the original substrate less toxic. However, the use of these traditional methods of treatment are disadvantageous since most of them require sophisticated equipment, time consuming and not eco-friendly [4].

The use of Effective Microorganisms in the treatment of wastewater is a promising technology that offers the advantages of cost effectiveness and eco-friendliness. It also requires less time. The original objective of the Effective Microorganism technology development was to enhance soil activity through the process of bioaugmentation and improve crop yield. This technology which has a wide array of application including agriculture, alga control, gardening, bioremediation and composting, was developed in the University of Ryukyus, Okinawa, Japan in the 1970's [5] [6]. The Effective microorganism technology is very promising and has great potential to create a suitable environment for the existence, propagation, and prosperity of biota [7].

2 Materials and Method

2.1 Sample Collection

Wastewater samples were collected from an oil field within River state in pre-sterilized bottles according Health Association [8] and transported immediately to the laboratory in an ice pack.

2.2 Isolation of Effective Microorganisms

The collected water samples were separately inoculated on selective media plates (Potato dextrose agar and De Man Rogosa and Sharpe agar) under aseptic condition. The obtained cultures were further sub-cultured by streak plate method on nutrient agar plates for bacterial colonies and potato dextrose agar for fungal isolates. The purified bacterial isolates were identified by morphological and biochemical characteristics based on Bergey's Manual of Determinative Bacteriology [9], while the fungal isolates were identified by microscopy and macroscopy.

2.3 Wastewater Treatment Using Effective Microorganisms

The inoculum was taken from the pure culture grown on agar slants using a sterile wireloop and inoculated into each test tube containing 5 ml of nutrient broth. The tubes were incubated at 37°C for 24 hours. 5 ml of the uniform suspension of

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each organism was inoculated as initial inoculum into each 500 ml Erlenmeyer flask containing 250 ml of sterilized sample. Samples were incubated for 3, 6, 9 and 12 days under room temperature. After desired incubation period the samples were analyzed for their microbiological and physicochemical parameters.

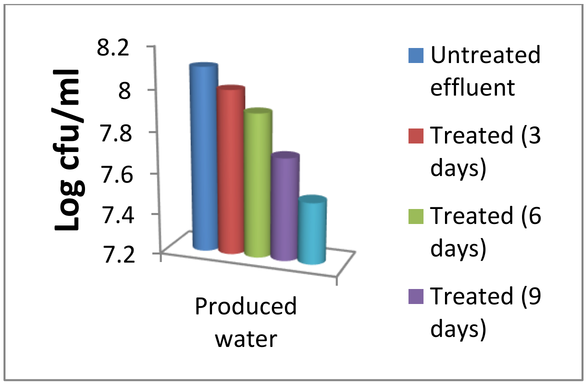

Figure 1: *Total viable bacterial count of untreated and treated produced water*

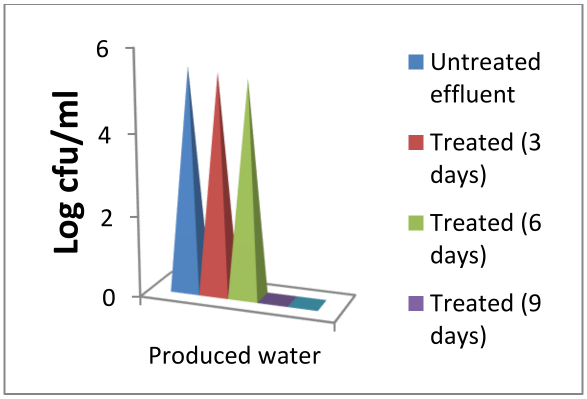

Figure 2: *Total Staphylococcal count of untreated and treated produced water*

2.4 Characterization of Wastewater Samples

Wastewater samples were characterized before and after the treatment. The spread plate techniques as described by American Public Health Association was used [8]. The prepared media were dispensed into sterile 9cm glass petri dishes and allowed to solidify. Duplicate plates were inoculated with 0.1ml aliquot of each dilution and spread using a flame sterilized hockey stick. Inoculated plate was incubated at 37°C for 24hrs. Morphologically different colonies were isolated and maintained at 4°C on nutrient agar slants. The purified isolates were identified by morphological and biochemical characteristics based on Bergey's Manual of Determinative Bacteriology [9].

3 Results

The total bacterial counts, staphylococcal counts and *Vibrio* counts of the treated water was shown to decrease as the period of treatment increased. The total heterotrophic bacterial counts (Figure 1) decreased from 8.1 Log cfu/ml to 7.5 Log cfu/ml, Staphylococcal counts (Figure 2) decreased from 5.5 Log cfu/ml to 0 Log cfu/ml and *Vibrio* counts (Figure 3) decreased from 1.9 Log cfu/ml to 1.3 Log cfu/ml. However, coliform counts (Figure 4) were shown to increase from 5.3 Log cfu/ml to 6.1 Log cfu/ml as the treatment period increased.

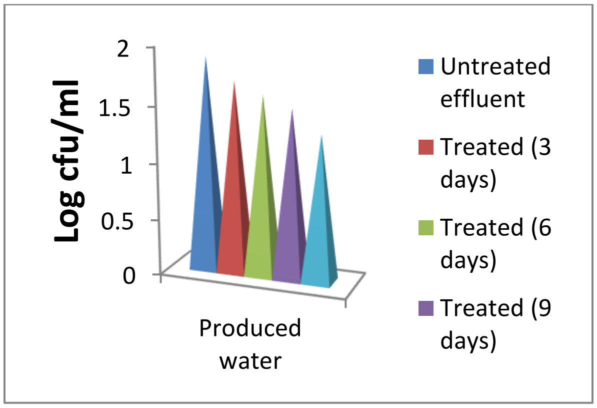

Figure 3: *Total Vibrio count of untreated and treated produced water*

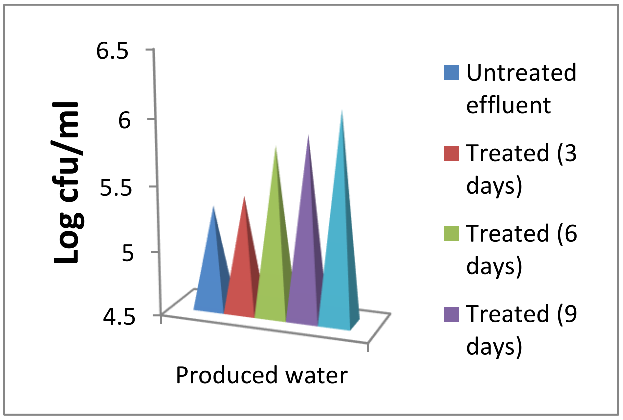

Figure 4: *Total coliform count of untreated and treated produced water*

Bacterial occurrence in treated and untreated effluent is shown in Table 1. *Bacillus* sp., *Klebsiella* sp, *Proteus* sp., *Escherichia coli* and *Staphylococcus* sp. were all present in the untreated produced water while *Enterobacter* sp was absent in both treated and untreated effluent. *Klebsiella* sp, *Proteus* sp. and *Escherichia coli* were the only organisms present in the treated effluent.

Table 1: *Bacterial occurrence in treated and untreated effluent*

Bacteria	**Untreated produced water**	**Treated produced water**
Enterobacter sp.	-	-
Bacillus sp.	+	-
Klebsiella sp.	+	+
Proteus sp.	+	+
Escherichia coli	+	+
Staphylococcus sp	+	-

Legends: + Present, - Absent

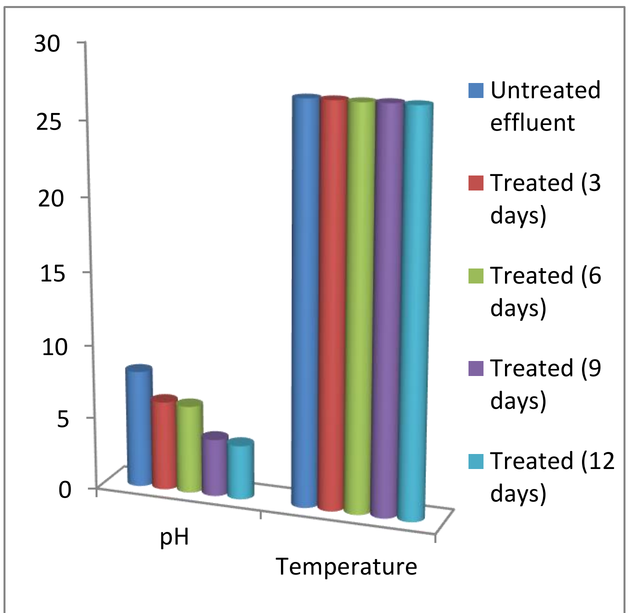

Figure 5: *pH and temperature values of produced water during treatment with effective microorganisms*

The pH and temperature ranges of the treated water are shown in Figure 5. The pH values of the produced water were steady as it decreased correspondingly while the temperature values fluctuated during the period of treatment. The pH for produced water decreased from 8.03 to 3.71 while the temperature values ranged between 26.98^oC to 27.03^oC. The BOD and COD values are shown in Figure 6. The values decreased correspondingly. The BOD values decreased from 240 mg/L to 21 mg/L while the COD values decreased from 400 mg/L to 160 mg/L.

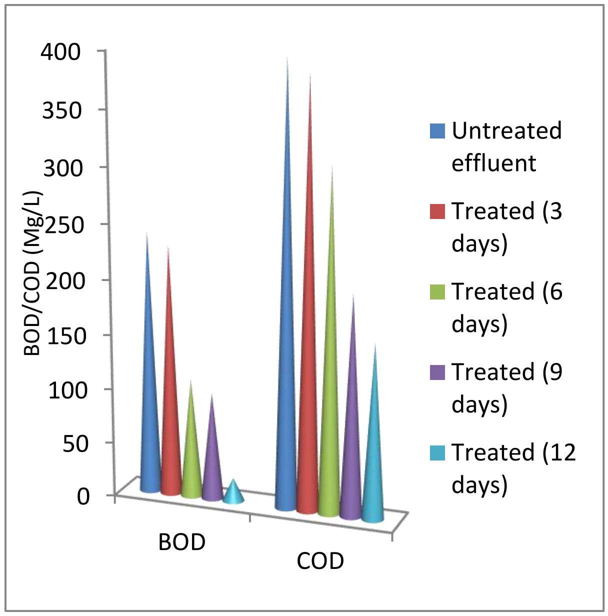

Figure 6: *BOD and COD values of produced water during treatment with effective microorganisms*

Legends: BOD – Biochemical Oxygen Demand; COD – Chemical Oxygen Demand

The concentrations of heavy metals (Zn, Fe, Ni, Cu and Cd) during the period of treatment are shown in Table 2. The concentration of Zn decreased from 0.113 mg/L to an undetectable level, Fe concentration decreased from 1.071 mg/L to 0.139, Ni values decreased from 2.110 mg/L to 1.081 mg/L while Cu and Cd were undetected in the wastewater samples. Selected standards of the heavy metal studied is shown in Table 3. The permissible limits of nickel for industrial wastewater discharged into surface water are 0.02, 0.05 and 0.02 mg/L respectively, while the permissible limits for iron are 1.0, 5.0 and 0.3 mg/L as stipulated by WHO (2007), FEPA (1999) and NSDWQ (2007) respectively.

Table 2: *Concentration of heavy metals in Produced water during treatment with effective microorganisms*

Parameter	Untreated	Treated (3 days)	Treated (6 days)	Treated (9 days)	Treated (12 days)
Zinc (mg/L)	0.113	0.100	0.051	< 0.01	< 0.01
Iron (mg/L)	1.071	1.038	0.911	0.212	0.139
Nickel(mg/L)	2.110	2.091	1.972	1.463	1.081
Copper (mg/L)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Cadmium (mg/L)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Table 3: *Some selected Standards of the Heavy Metals studied (mg/l)*

	Iron	Zinc	Nickel	Copper	Cadmium
WHO	1.0	<1	0.02	0.05	0.03
FEPA	5.0	<1	0.05	0.01	0.05
NSDWQ	0.3	<1	0.02	1.0	0.03

LEGENDS: WHO - World Health Organization; FEPA - Federal Environmental Protection Agency; NSDWQ-National Standard for Drinking Water Quality

4 Discussions

Results of the treatment analysis revealed a corresponding decrease in the total viable bacterial count as the treatment/incubation period increased from1.3 x 10^8 cfu/ml to 3.6 x 10^7 cfu/ml for produced water. Similarly, counts of *Staphylococcus* and *Vibrio.* spp. decreased as the incubation period increased. Initial counts were observed to be 3.4 x 10^5 cfu/ml and 8.0 x 10^5 cfu/ml for *Staphylococcus* and *Vibrio.* spp. respectively, subsequently there was a reduction in counts and absence of growth was observed on the 12th and final day of incubation. The reduction in bacterial count could be attributed to the characteristic antimicrobial properties possessed by lactic acid bacterial specie which was used for wastewater treatment. The mechanism of action of probiotics with anti-microbial properties maybe due to the production of bacteriocins such as nicin or lowering the pH by producing acidic compounds like lactic acid [10]. However, a corresponding increase in total coliform count was observed as the treatment period increased. Counts increased from 2.0 x 10^5 cfu/ml to 1.4 x 10^6 cfu/ml for produced water. Acid adaptation and increasing resistance to acidic conditions have been observed for various coliform organisms including *Salmonella* and *E. coli* [11]. The ability to survive under extreme acidic conditions of human stomach, greatly contributes to the virulence of all the enteric human pathogens [12].
The results of the physicochemical analysis carried out revealed pH values of produced water reduced as the incubation period increased with values from 8.03 - 3.71. The reduction in pH could have been as a result of the organic acids produced by lactic acid bacteria specie. Studies have demonstrated the production of lactic acid and acetic acid by lactic acid bacteria and consequently leading to a decrease in pH [13]. The decrease in pH observed in the study corresponds with the decrease in bacterial population observed earlier thereby giving a strong indication that the acidic environment created by the production of organic acids eliminated the physiological group of bacteria that could not survive a very low pH environment.

The temperature of the effluents was observed to fluctuate slightly as the treatment period increased. The temperature of the wastewater ranged from 26.98 to 27.03 ^oC for produced water. The fluctuation in temperature values can be attributed to the ambient temperature during incubation.

The biochemical oxygen demand (BOD) values of wastewater samples were observed to decrease as the incubation period increased. BOD values of produced water decreased from 240.0 to 21.0 mg/L. In a similar study carried out by Sonune and Garode [14] on the bioremediation potential of bacterial isolates for municipal wastewater treatment, there was a reduction in the BOD values from 48.91 to 42.86% after the period of treatment with microorganisms. Similar results were observed by Shrivastava *et al.* [15] and Prasad and Manjunath [16] who reported that *B. subtilis* and *Pseudomonas aeruginosa* has BOD and COD reduction potential while studying the bioremediation of Yamuna water and lipid rich wastewater respectively.

The Chemical oxygen demand (COD) values were similarly observed to decrease with an increase in incubation period. COD values decreased from 400.0 mg/L to 160.0 mg/L for produced water. The reductions of COD and BOD concentrations could be facilitated by activities of microorganisms that can break down the organic compounds either suspended or dissolved in the wastewater.

Results of the heavy metal analysis revealed the presence of zinc, iron and nickel in the produced water sample while copper and cadmium were absent. The initial heavy metal (nickel and iron) concentration in the wastewater samples were above the permissible limits according to WHO [17], FEPA [18] and NSDWQ [19]. The permissible limits of nickel for industrial wastewater discharged into surface water are 0.02, 0.05 and 0.02 mg/L respectively, while the permissible limits for iron are 1.0, 5.0 and 0.3 mg/L as stipulated by WHO [17], FEPA [18] and NSDWQ [19] respectively. These values however reduced and fell within the threshold limit after the wastewater sample was treated. The initial concentration of zinc was revealed to be within permissible limit. The high rate at which these metals are used in industries have greatly affected the quality of wastewater generated from the companies [20]. These metals are regularly taken up by microorganisms with the requisite enzymes and they bioaccumulate within the system [21]. The monitoring of heavy metal removal revealed a decline in the heavy metal concentration as the treatment period progressed. The concentration of zinc decreased from 0.113 to $<$ 0.001 mg/L, iron concentration decreased from 1.071 to $<$ 0.001 mg/L, nickel concentration decreased from 2.110 to 1.081 mg/L. In a similar study carried out by Olukanni *et al.* [22] on biosorption of heavy metals in industrial wastewater using microorganisms, a significant reduction in the concentration of heavy metals (cadmium, zinc and silicon) in the wastewater was observed. The ability of microorganisms to uptake metals can also be attributed to the presence of metal binding proteins such as MTs and PCs which are capable of metal adsorption and accumulation within the cell [23].

5 Conclusions

The results of the study have shown that a wide range of bacteria that could possibly be pathogenic are present in the produced water studied. The presence of these organisms is a public health concern and therefore efforts should be made to rid wastewaters of these pathogens. The presence of heavy metals in the study also pose health risk to users and inhabitants of the receiving water bodies. They are toxic and can bioaccumulate in aquatic organisms, affecting man via food chain. The study further revealed that effective microorganisms such as *Aspergillus* sp, *Penicillium* sp and *Lactobacillus plantarum* (used for treatment in the study) are effective in the reduction of pathogenic microorganisms, BOD, COD and removal of heavy metals (Zinc, Iron and Nickel) to an undetectable level. The heavy metals studied fell within the permissible limits set by regulatory bodies. This process of bioremediation is safe, cost effective and also environmental friendly. Efforts should be intensified by regulatory bodies to monitor and ensure proper and safe disposal of effluents. Due to its cost effectiveness and environmental friendly advantage, wastewater producing industries should embrace the use of effective microorganisms in the treatment of wastewater.

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