

Bacteriological and Physicochemical Assessment of Treated Petroleum Effluent Obtained from Some Petrochemical Industries in Warri, Nigeria Using Activated Rice Husk

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Abstract This study assessed the efficacy of activated rice husk in the reduction of pollutants from petroleum effluent. The effluent sample was collected from an effluent discharge from a petroleum processing company, and transported to the laboratory for analyses. The most effective treatment was observed with sample T3, with the highest dosage of activated rice husk. BOD and COD of sample T3 reduced from 53.17 mg/l and 112.48 mg/l to 8.83 mg/l and 19.14 mg/l, while total hydrocarbon and Oil and Grease reduced from 142.931mg/l and 148.426 mg/l to 9.063 mg/l and 10.425 mg/l. Microbial load after treatment include T1 (10g treatment) *Staphylococcus aureus* (2.5×10^6), *Bacillus subtilis* (2.9×10^6), *Clostridium botulinum* (2.4×10^5), *Pseudomonas aeruginosa* (3.5×10^4), *Proteus vulgaris* (4.5×10^5), *Escherichia coli*, (2.4×10^6) and *Klebsiella pneumonia* (3.2×10^4). T2 (50g treatment) after treatment microbial load include *Staphylococcus aureus* (2.2×10^6), *Bacillus subtilis* (2.1×10^4), *Clostridium botulinum* (1.4×10^5), *Pseudomonas aeruginosa* (1.5×10^4), *Proteus vulgaris* (2.5×10^5), *Escherichia coli*, (2.3×10^6) and *Klebsiella pneumonia* (1.6×10^6). T3 (100g treatment) after treatment microbial load include *Staphylococcus aureus* (2.0×10^4), *Bacillus subtilis* (1.8×10^6), *Clostridium botulinum* (1.2×10^5), *Pseudomonas aeruginosa* (1.4×10^6), *Proteus vulgaris* (1.3×10^4), *Escherichia coli*, (1.5×10^4) and *Klebsiella pneumonia* (1.5×10^5). T1 microbial load ranged from 2.5×10^6 - 4.5×10^5 , with *Proteus vulgaris* having the highest, while *Staphylococcus aureus* having the lowest. For T2 (50g treatment) after treatment microbial load ranged from 1.4×10^5 - 2.5×10^5 with *Clustridium butilinum* having the highest microbial count, while *Proteus vulgaris* had the lowest count and, for T3 after treatment microbial load ranged from 1.2×10^5 - 2.0×10^4 , with *Staphylococcus aureus* having the highest while *Clustridium butilinum* having the highest microbial count. The reduction in the microbial population and physicochemical properties after treatment revealed that rice husk is effective in the treatment of petroleum effluent before being discharged into the water bodies.

Keywords: petrochemical effluents, potential bioabsorbent, rice husks, microbial growth effluent discharge

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1. Introduction

Water, a substance composed of the chemical elements hydrogen and oxygen and existing in gaseous, liquid, and solid states. It is one of the most plentiful and essential of compounds. A tasteless and odourless liquid at room temperature, it has the important ability to dissolve many other substances [1]. Indeed, the versatility of water as a solvent is essential to living organisms. Life is believed to have originated in the aqueous solutions of the world's oceans, and living organisms depend on aqueous solutions, such as blood and digestive juices, for biological

processes. Water also exists on other planets and moons both within and beyond the solar system. In small quantities water appears colourless, but water actually has an intrinsic blue colour caused by slight absorption of light at red wavelengths. Water serves numerous function which include drinking, as biochemical solvent, irrigation, transport industries [2].

Contaminated water and poor sanitation are linked to transmission of diseases such as cholera, diarrhoea, dysentery, hepatitis A, typhoid and polio. Absent, inadequate, or inappropriately managed water and sanitation services expose individuals to preventable health risks. This is particularly the case in health care facilities where both patients and staff are placed at

additional risk of infection and disease when water, sanitation and hygiene services are lacking [2]. Globally, 15% of patients develop an infection during a hospital stay, with the proportion much greater in low-income countries.

Inadequate management of urban, industrial and agricultural wastewater means the drinking-water of hundreds of millions of people is dangerously contaminated or chemically polluted. Natural presence of chemicals, particularly in groundwater, can also be of health significance, including arsenic and fluoride, while other chemicals, such as lead, may be elevated in drinking-water as a result of leaching from water supply components in contact with drinking-water [3].

Oil and gas industry depends heavily on water in many different aspects. Wherever there is oil or gas production, water is present. Therefore, understanding the properties and composition of the water in petroleum industry is essential. Unfortunately, water in petroleum industry does not get as much attention as the hydrocarbon fluids from researches and studies efforts. Water is a two edged sword. It can enhance the production and profitability of the development plan or be a direct reason of losing production and effectiveness of the investments [2].

Excessive water production is one of the main well-known problems that faces most of the oil operators worldwide in the hydrocarbon industry. Although it is a classic difficulty that occurs in old developed fields, it can happen in new ones too [1]. It causes numerous economic problems to the oil and gas production companies. First, excessive water affects the performance of the production wells and shorten their life period. The presence of the water in the wellbore increases the weight of the fluid column, which leads to an increase in the lifting requirements. That increases the operating cost and leads to a lower drawdown. For example, if the well is a gas lifted, the amount of gas injected to lift the fluid column from the wellbore to the surface is higher with the production of excessive water than without producing it. Water production also enhances the presence of scales, corrosions and degradation in the field facilities starting from the wellbore completions to the surface facilities [4]. Another major problem is the cost of separating, treating and disposing the produced water, which is a great burden over the oil company budgets. It costs around \$1 Billion/year in Alberta to dispose the produced water [5] getting rid of those kind of problems help in reducing expenses for the operators and increase the profitably of their operations [4].

On the other hand, water is one of the most important drives for oil production since it helps in managing the pressure of the reservoir, mobilizing the oil and displacing it in the homogenous rocks. This water is known by necessary or good water production. This water is typically associated with oil production in the late stages of water-flooding operations or from active aquifers. It is also the water produced at a low Water Oil Ratio (WOR) which maintain the profitably of a production well [6]. Attempts to reduce this kind of water production leads directly to reduction in the oil production [7]. On the contrary, the un-wanted water production is the type, which needs to be eliminated and reduced in order to increase the productivity and the profitability of the production wells [8]. Operators focus on eliminating

unwanted water production, which is also named as Bad Water. This kind of production creates problems, other than the mentioned previously, such as losing oil production and poor sweep efficiency within the matrix rocks. That means simply, losing money! The worst problem among unwanted water production issues is the un-swept areas and oil pockets that are left behind as a result of bad conformance jobs. This case is commonly known in water-flooding operations where water is simply injected through injection wells to displace hydrocarbons toward the production wells and maintain the pressure of the reservoir. In many cases, the water goes to an open fracture or high permeability layer since it is all about the resistance of the paths in the reservoirs. The least resistance path is the winner in attracting the injected water toward it and the oil in the matrix rock stays behind without achieving the required sweep to attain efficient oil sweeping or good conformance [7]. If the production well happens to be connected to the open fracture or the high permeability layer, unwanted water production would occur. It is essential to be able to differentiate between those two types of water production in order to maintain the productivity of the well. One of the ways to identify the type of the excessive water production in a certain well is by studying the offset wells water cut behavior. It is a bad water production if the offset wells are producing with much lower water cut [9].

As mentioned previously, many reservoirs worldwide are water drive. In addition, operators use water to provide pressure support artificially to the production by injecting it into the reservoir. That gives us two main kinds of water in the petroleum industry, Produced water and Injected water. Their properties and composition can contrast broadly. Water presence can be also a nightmare for the operators [2] Water is the main source of scale deposits and corrosion that occurs in the upstream and downstream equipment. For the reservoir, it can form emulsion, creates skin and plug the formation. Those problems caused by water usually lead to huge expenses and reduce the profitability of the operation. That can be controlled if operators understand the properties of the water in their field and take pro-active steps to minimize the damage caused by water to the facilities and the reservoirs [10].

Petroleum refinery effluents (PRE) are generally the wastes generated from industries primarily engaged in refining crude oil, manufacturing fuels, lubricants and petrochemical intermediates. These effluents or wastewater, generated, are considered as a major source of aquatic environmental pollution. The effluents are mainly composed of oil, grease and many other toxic organic compounds [11]. The process of crude oil refining consumes large volume of water. Consequently, significant volume of wastewater is generated. The requirement of water depends upon on the size, crude products and complexity of operation. Petroleum refining units need water for distillation, desalting, thermal cracking, catalytic and treatment processes in order to produce useful products such as LPG (Liquefied Petroleum Gas), gasoline, asphalt, diesel, jet fuel, petroleum feedstock [12]. Wastewater generated through petroleum refineries contains various hydrocarbons. It has been estimated that the demand for world oil is expected to rise to 107 mbpd (million barrels per day) in the next

two decades. By 2030 oil will account for 32% of the world's energy supply. The increasing demand of oil clearly shows that effluents produced from the oil industry will continue to be produced and discharged into the water bodies. The pollutants found in the effluent are seriously toxic and hazardous to the environment. Techniques used for effluent treatment include adsorption, coagulation, chemical oxidation, biological techniques as well as contemporary technologies like membranes and microwave-assisted catalytic wet air oxidation and Advanced oxidation processes (AOP) like heterogeneous photo-catalytic degradation which is based on its potential to completely mineralize the organic effluents beside being cost effective, readily available and the catalyst used itself is non-toxic in nature [10].

Petrochemical effluents contain a large amount of suspended solids, organic matter, oil and grease, sulphide, ammonia, phenols, hydrocarbons, benzene, toluene, xylene, and polycyclic aromatic hydrocarbons [2,13]. Industries such as petroleum refining and tank farms, produce large quantities of effluents from several processes including desalting, atmospheric and vacuum distillations, hydrocracking, catalytic cracking and catalytic reforming as well as alkylation [14].

The residuary water produced in oil refining plants is a threat to quality of water bodies downstream of the discharge point [2]. Following industrial revolution experienced world-wide which has brought with its technological development the growth of pollution has since assumed large proportions. Many natural organic, inorganic, and artificial compounds are present in petroleum effluent; Inorganic and organic compounds are usually biodegradable that can be metabolized by microorganisms which reduces the oxygen available for other life forms [3,15]. The wastewater surroundings are a perfect medium for both pathogenic and nonpathogenic microorganisms; dangerous pathogens include enteric bacteria, viruses, protozoa, parasitic worms [16]. An increased level of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) depletes the amount of available oxygen for organisms inhabiting the environment [11]. The estimation of heterotrophic bacteria as an indicator of water quality and accurate taxonomic identification of the bacterial community is made possible by extracting and analyzing its total nucleic acids [17].

Environmental pollution has become a major concern in developing countries in the last few decades, main sources of water contamination are the untreated or partially treated industrial effluents and the petroleum refining industry among others is known globally as a major actor in water pollution process [18]. Ignorance of the harmful effects of discharging wastewater from these processes directly into the environment untreated has been identified as a major problem [19]. The noxious wastes present in petrochemical effluent can only be partially eliminated by conventional treatment processes that are applied [9].

Advanced treatment techniques such as membrane processes and adsorption are the major unit operation used for the removal of various pollutants from effluent, which offers flexibility in the selection of contacting devices and adsorbent material depending on the time, space and funds available for treatment of the effluent [20]. These

processes are expensive and time consuming however, advanced technologies for oily wastewater treatment called microbial bioremediation technology an emerging and state-of-the-art technology that employs metabolic potential of microorganisms for the removal of hazardous pollutants from oily wastewater under aerobic or anaerobic conditions, or a cocktail of both through complete degradation or sequestration [12,21,22]. The microbes use the pollutants as carbon source and convert them into innocuous products through secretion of suitable metabolites [23,24,25]. The microbial activity depends on parameters such as temperature, pH, toxic elements, presence or absence of oxygen, moisture, redox potential, retention times and organic contents [26,27].

Rice husk is a protective layer from the rice grains processing, the major wastes gotten from rice processing is, it contains cellulose (32.24%), hemicelluloses (21.34%), lignin (21.44%), water (8.11%), extractives (1.82%) and mineral ash (15.05%) as well as high percentage of silica in its mineral ash [28,29]. Several studies have proven the efficacy of rice husk as an adsorbent which depends on dosage, contact time, and temperature [30]. However, not much works have been done on the treatment of industrial wastewater with rice husks [31]. Most researchers have targeted the use of agro-waste based activated carbon in the removal of only one component or the other that form part of the pollutants from simulated refinery effluent [31]. In this light, this research targets the treatment of Petroleum Effluent with rice husks - a case study of oil processing company (Matrix Energy Limited) in Ubeji area of Warri, Delta State.

2. Materials and Methods

2.1. Sample Collection, Preparation and Treatment of Effluent

The effluent was collected from an effluent discharge point of a petroleum processing company, Matrix Energy, located in Warri Delta State. Samples were collected in ten liters (10 L) can, preserved with ice chest, and transported to the Delta State University Laboratory for microbial and physicochemical analysis.

2.2. Rice Husk Collection and Preparation of Adsorbent (Activated Rice Husk - ARH)

The rice husk was collected from a rice mill situated in Badagry area of Lagos State. The rice husk was screened, washed, dried in an oven at 60°C for 2 hours. It was thereafter soaked in 1.0 mol/l of Zinc chloride for an hour, rinsed for about 2-3 times with distilled water, oven dried at 105°C for 1 hours and was then sieved through BSS-30 mesh size particle.

2.3. Treatment of Effluent with Different Adsorbent Doses (ARH)

Three (3) doses of Activated Rice Husk (ARH) made up of 10 g, 50 g and 100g of rice husk, were prepared and

labelled T1, T2 and T3 respectively. ARH were weighed into different conical flasks containing the same volume of industrial effluent of 1000 ml. The flask with Activated Rice Husk ARH was agitated at a speed of 2000 rpm for 30 minutes and were left for seven (7) days to allow the activated rice husk to properly absorb the pollutants from the effluent. The Adsorbent was then filtered to get the clear samples for analysis.

2.4. Determination of Physicochemical Properties of the Effluent

Physicochemical properties of the effluent that was investigated include; pH, total dissolved solid, electrical conductivity, turbidity, dissolved oxygen, biochemical oxygen demand (BOD) total hydrocarbon content (THC), determination of oil and grease (O&G), pH and temperature was measured using electrometric method (APHA 460), and the pH value was taken and recorded. The temperature value displayed alongside with the pH value. Total dissolved solid, electrical conductivity, and turbidity, were determined using electrometric method (APHA 145), chemical oxygen demand (COD) and biochemical oxygen demand (BOD) (APHA 507) value was taken and recorded. Dissolved oxygen was determined using Winkler's method. Chemical oxygen demand (COD) was determined using open reflux method (APHA 508/ASTM D1252), total suspended solids filtration technique (APHA 208D/ASTM D1868) were the weight difference between a preweighed filter paper and the weight of the filter paper were determined after drying at 105°C. Total hydrocarbon content (THC) was determined by solvent extraction method (ASTM D3921/APR-RP 206). Oil and grease (O&G) was determined by gravimetric method (APHA 5520B) were the processed and purified samples were distilled in water bath at 85°C till visible condensation stops. Then it was cooled in a desiccator until a constant weight was observed. The sample volume was calculated by the difference between the initial weight of flask and total weight of flask and residue, minus tare weight of flask and initial sample volume.

2.5. Bacteriological Analyses

An accurate aliquot (1 mL) of the effluent samples before and after treatment was suspended in 9 mL sterile water and mixed for 4 min at 2000 rpm. Serial dilution was done on the resulting suspensions, 0.1 mL from the 10-1 dilution of the sample were spread onto plates containing nutrient agar medium, m. endo agar, m-fc agar, centrimide agar, manitol salt agar, m.enterococcus agar, eosin methylene blue agar, manitol salt agar and macConkey agar, incubated for 24h- 48h at 37°C, and the number of colonies were counted using digital colony counter.

2.6. Identification of Bacterial Isolates

Isolates were characterized using standard tests which include; Gram reaction and shape, catalase, motility, bile solubility test, aerobic growth, anaerobic growth, coagulase, oxidase, hydrogen sulphide production, decarboxylase, citrate utilization, urease, deoxyribonuclease, phenylalanine deaminase, indole, methyl red and sugars fermentation.

3. Results and Discussion

3.1. Physicochemical Properties of the Oily Effluent before Treatment

The physicochemical properties of the oily effluent before treatment was carried out and the result is presented as [Table 1](#). The physical parameter namely the pH, temperature, total dissolved solid, electrical conductivity, were all observed to be higher than the acceptable limit set the Department of Petroleum Resources and world health organization [32]. These values exceeded the acceptable limit for oily effluent discharge as recommended by DPR regulatory standard. The absence of Dissolved Oxygen, the objectionable odour and high turbidity further confirms the pollution level of the effluent.

The physicochemical properties of the oily effluent after treatment is presented in [Table 2](#). pH of the treated effluents of samples T1, T2, and T3 conformed to the DPR standard of 6.5-8.5.

Table 1. Physicochemical Properties of Oily Effluent before treatment (Pre- treatment)

Parameters	Units	Effluent (Raw sample)	DPR Limit
Ph		10.1	6.5 - 8.5
Temperature	°C	32.7	25 – 35
Total Dissolved Solid (TDS)	mg/l	10341.00	<2000.00
Electrical Conductivity (EC)	µS/cm	14273.00	N/A
Odour		Objectionable	Unobjectionable
Turbidity	N.T.U	26.15	N/A
Dissolved Oxygen (DO)	mg/l	ND	>4.00
Total Suspended Solid (TSS)	mg/l	60.00	30.00
Biochemical Oxygen Demand (BOD)	mg/l	53.17	10.00
Chemical Oxygen Demand (COD)	mg/l	112.48	40.00
Total Hydrocarbon Content (THC)	mg/l	142.931	10.00
Oil and Grease (O & G)	mg/l	148.426	10.00

Table 2. Summary of Physicochemical Properties of Oily Effluent both Before and After Treatment

Parameters	Unit	UT	T1	T2	T3	DPR LIMIT
pH		10.1	7.63±0.32a	7.2±0.100a	6.6±0.100a	6.5-8.5
Temperature	°C	32.7	32.4±0.36a	30.66±0.47a	30.60±0.100b	25-35
Total Dissolved Solid	mg/l	10341	8631±1.00a	8634±1.00a	8941±1.00a	<2000.00
Electrical Conductivity	µS/cm	14273	11550±1.00a	11860±0.57a	12049±5.27a	<2000
Odour		Unobj	Unobj	Unobj	Unobj	Unobj
Turbidity	N.T.U	26.15	3.28±0.057a	2.47±0.057a	1.93±0.057a	10-15
Dissolved Oxygen	mg/l	ND	.90±0.010a	4.60±0.010a	5.90±0.010a	>2.0
Total Suspended Solid	mg/l	60	10.00±0.20a	5.00±0.100a	3.03±0.057a	<30
BiochemicalOxygen demand	mg/l	53.17	18.17±0.01a	13.42±0.01a	8.83±0.010a	10
Chemical Oxygen Demand	mg/l	112.48	42.59±0.010a	29.25±0.01a	19.14±0.010a	40
Total Hydrocarbon Content	mg/l	142.931	58.33±0.01a	36.92±0.010a	9.08±0.02a	10
Oil and Grease	mg/l	148.426	62.81±0.01a	38.17±0.02a	10.00±0.01a	10

Key: UT= Untreated Effluent, T1= Treated Effluent 1 (10g treatment), T2= Treated Effluent 2 (50g treatment), T3= Treated Effluent 3 (100g treatment). Values are represented as mean ± standard deviation, of three replicates in all groups. Values with different superscript are significantly different at (p<0.05).

The gradual reduction in pH of the treated effluent could be as a result of chemical composition of the absorbent (ARH) used for the treatment, while the alkalinity nature of the untreated effluent could have resulted from organic composition of the effluent. In biosorption studies, pH influences the surface charge of the biosorbent, degree of ionization of the organic substances and the dissociation of functional groups. This is in conformity with the report by [33] which says oil industry effluent is characterized by alkaline pH.

The temperatures (°C) of the treated effluent for samples T1, T2 and T3 also conformed to the DPR standard limit of 25 - 35°C for industrial effluent discharge [32]. The range observed could have been due to the aeration condition during analysis. When effluents within these temperature ranges are discharged into water bodies will have little or no influence on the aquatic habitat and its inhabitants. The temperature of surface water governs to a large extent, the biological species present and their rates of activities. Higher order species, such as fish, are affected dramatically by temperature and DO levels which are a function of temperature. The observed temperature range of 25 - 35°C is consistent with that reported by [34].

The turbidity values of the samples were lower than the 15 NTU DPR limits (Table 2). This indicates that the treatment was effective in ameliorating this effects. This may have to do with the nutrient load of the effluents. The highest value of 26.15 NTU was recorded in the untreated sample.

Turbidity being an important factor in mixing and transport of nutrients and waste product in water, some organisms depend entirely on this processes for survival. The high value observed in the untreated sample was a pointer to the high pollution level of the industrial effluent. This is confirmed by the report of [35] which says that oil polluted water is highly turbid.

High turbidity can significantly reduce the aesthetic quality of lakes and streams, having a harmful impact on recreation and tourism. It can increase the cost of water treatment for drinking and food processing. It can harm fish and other aquatic life by reducing food supplies, degrading spawning beds, and affecting gill function. A report of the European Inland Fisheries Advisory Commission lists five ways that fine particles can have a

harmful impact on freshwater fish: acting directly on fish, killing them or reducing their growth rate, resistance to disease, etc., preventing successful development of fish eggs and larvae; modifying natural movements and migrations; reducing the amount of food available; and affecting the efficiency of methods for catching fish.

The DO levels showed a wide range of fluctuation between 3.90 and 5.90 mg/l. DO levels of below 2.50 mg/L are indicative of environmental stress. The values observed in the effluents in this study were in conformity to the DPR set standard, thus they are capable of sustaining marine/estuarine life when discharged into the water bodies however, lack of oxygen in an effluent or surface water signifies pollution [9]. Reduced DO of any sample may be due to the organic pollutant dissolved in the oily effluent [9]. The treated effluents were moderately oxygenated as it shows compliance with DPR acceptable limit of >2.00mg/l [32].

TSS, which is the filterable particles in the effluent, had values 3.90 to 10.00 mg/L) which was within the recommended DPR standard (<30 mg/L). High TSS in the untreated effluent is an indication of high pollution and can cause an increase in the surface water temperature, because the suspended particles absorb heat from sunlight when discharged into water bodies. This could be as a result of the effective effluent treatment capability as observed during the study.

Total dissolved solid is a measure of the amount of mineral salt present in water sample, the values gotten were observed to be above the DPR set standard of < 2000. Both untreated and treated effluent were far above the DPR set standard. This shows that the load of mineral salt dissolved in the effluent was far more than. However, there was a reduction of the absorbed mineral salts from 10341 that observed in the untreated effluent to 8631±1.00^a for sample T1, 8634±1.00^a T2, and 8941±1.00^a for T3.

Total dissolved solids are usually comprised of inorganic salts and a small portion of organic matter that provide benefits such as nutrients or contaminants such as toxic metals and organic pollutants. The types of inorganic salts that dissolve in the water include sulfates, potassium, calcium, magnesium, chlorides, and bicarbonates. TDS concentrations should be at a certain level for every type of water. Current regulations require the periodic

monitoring of TDS, which is a measurement of inorganic salts, organic matter and other dissolved materials in water. Measurements of TDS do not differentiate among ions.

TDS is often monitored in order to create a water quality environment favorable for organism productivity. For freshwater oysters, trouts, and other high value seafood, highest productivity and economic returns are achieved by mimicking the TDS and pH levels of each species' native environment. For hydroponic uses, total dissolved solids is considered one of the best indices of nutrient availability for the aquatic plants being grown. A number of studies have been conducted and indicate various species' reactions range from intolerance to outright toxicity due to elevated TDS. The numerical results must be interpreted cautiously, as true toxicity outcomes will relate to specific chemical constituents. Nevertheless, some numerical information is a useful guide to the nature of risks in exposing aquatic organisms or terrestrial animals to high TDS levels as observed in this study.

The electrical conductivity of both untreated effluent and treated effluent were observed to be above the DPR set standard of <2000. The conductivity of a medium is an indication of its ability to conduct an electric current. It is usually assessed by the presence of total concentration of ions, temperature, etc. Conductivity values higher than the 2000 $\mu\text{S}/\text{cm}$ were obtained from all the effluent samples. These high conductivities results might be due to increased decomposition and mineralization of some organic matter. Higher conductivity range from 11550 to 12049 $\mu\text{hos}/\text{cm}$ was obtained from all the effluent samples. Only ionized substances contribute to conductivity of water [34].

The biochemical oxygen demand (BOD) (mg/l) is a measure of the oxygen requirement of effluents and polluted waters for the biochemical degradation of organic materials and the oxygen used to oxidize inorganic material, such as sulphides, ferrous ions, and nitrogen. It's a test for the quantity of oxygen utilized during a specific incubation period as well as the quantity of oxygen used to oxidize inorganic material [9].

The BOD value of over 10.00mg/l in oily effluent indicates some level of pollution [32]. The observed values of BOD₅ was significantly within the acceptable limits in sample T3 (8.83). This suggests that most available oxygen is used for biodegradation of waste within the environment; hence, very small amount was left for biochemical activities. However, BOD₅ values of sample T1 and T2 were observed above the set standard. BOD₅ depicts the amount of putrescible organic matter degradable by microbial metabolism on the assumption that the water has no bactericidal or bacteriostatic effects. BOD and COD concentrations are indicators of the level of organic compounds in polluted water [36]. Excessive level of BOD and COD may result in low levels of dissolved oxygen and can be detrimental to fishes and inhibit the reproduction rates of aquatic organisms [37].

Chemical oxygen demand (COD) (mg/l) is used as a measure of the oxygen equivalent of the organic matter content of water that is susceptible to oxidation by a strong chemical oxidant. COD value of over 40mg/l indicates pollution [32]. However, sample T2 (50 g treatment) with value of 29.25 ± 0.01^a and sample T3 (100g treatment) 19.14 ± 0.010^a conformed to DPR acceptable

limit of 10.00mg/l for effluent discharge. This indicate that the higher the activated rice husk the more efficient is the treatment process. This agrees with the report of [38] which says organic pollutants produced by industrial activity such as hydrocarbons can be gradually removed by bioremediation.

Basically, oil and grease composed of hydrocarbons of both anthropogenic/petrogenic and biogenic origins. THC is an index to measure the carbon-containing compounds in a medium. It serves as a means of determining the level of organic contamination in a given environment. [39] reported that high organic carbon content increases the growth of microorganisms which then leads to the depletion of oxygen supply.

Total hydrocarbon content (THC) of untreated, T1 and T2 effluent samples were above detectable limits which implies that the treated samples still contain certain amount of hydrocarbons. However, treatment process have been able to reduce it significantly from 142.931 mg/L to 42.59 in sample T1, 36.92 in sample T2 respectively. This is attributable to the very effective effluent treatment process using activate rice husk.

However, sample T3 (9.08 mg/L) conformed to DPR acceptable limit of 10.00mg/l for effluent discharge. According to [40], hydrocarbon in wastewater can cause depletion of dissolved oxygen and loss of biodiversity in the receiving water bodies.

The concentrations of oil and grease in wastewater streams have been observed to increase adverse effects on the ecology. This results from the increasing oil and grease use as well as indiscriminate discharge of oil and grease into the water drains, domestically and industrially.

Meanwhile, the oil and grease content of the treated effluents was found to be ranging from 62.81 ± 0.01^a for sample T1 (10g treatment), 38.17 ± 0.02^a for sample T2 (50g treatment) to 10.00 ± 0.01^a for sample T3 (100g treatment), while untreated effluent was 148.426. This implies that the treated samples still contain certain amount of oil and grease. However, the treated sample T3 (100g treatment) conformed to DPR acceptable limit of 10.00mg/l for effluent discharge.

This study reported the applications, efficiencies, and challenges of oil and grease wastewater treatment from industrial wastewater and municipal water stream. The results showed that the concentrations of oil and grease discharged into the ecosystem lead to increase environmental impact. The desired development for effective removal of oil and grease is discussed as emerging pollutants.

The efficiency analysis of the treated effluent in the area of oil reduction is presented in Table 3. From the results, it can be deduced that removal of pollutants has been observed with the increase in ARH dosage. In this study, the percentage treatment value of 88.25% for treated sample T3 shows the most effective treatment. This is because the treatment was able to absorb the highest amount of pollutants from the effluent. The parameters such as BOD, COD, THC and O&G, being the pollution indicators were used for the calculation of percentage treatment.

This study has demonstrated the effectiveness and absorption capacity of rice husk in the treatment of aqueous solution and industrial effluents. Thus a potential bioabsorbent for toxic industrial effluents

Table 3. Percentage Treatment of Samples

Parameters	UT	T1	%treatment	T2	%treatment	T3	%treatment
BOD	53.17	18.17+0.0 ^a	65.83	13.42+0.0 ^a	74.76	8.83+0.01 ^a	83.39
COD	112.48	42.59+0.0 ^a	62.14	29.25+0.0 ^a	73.99	19.14+0.0 ^a	82.98
THC	142.91	58.33+0.0 ^a	59.18	36.92+0.0 ^a	74.17	9.08+0.02 ^a	93.66
O&G	148.46	62.81+0.0 ^a	57.67	38.17+0.0 ^a	74.29	10.00+0.0 ^a	92.98
Total %Treatment			61.2		74.3		88.25

Key: UT= Untreated Effluent, T1= Treated Effluent 1 (10g treatment), T2= Treated Effluent 2 (50g treatment), T3= Treated Effluent 3 (100g treatment). Values are represented as mean $\pm$ standard deviation, of three replicates in all groups. Values with different superscript are significantly different at ($p < 0.05$).

3.2. Microbial Parameters

The microbial population, cultural, morphological, and biochemical characteristics of bacterial isolates from untreated and treated effluent sample with activated rice husk for the various samples is presented in Table 4 and Table 5. Results from the study confirmed that the incidence of microbial population could be due to factors such as nutrient, minerals, temperature, oxygen level, acidity, volume of wastewater concentration of oil and grease constant exposure to hydrocarbon component could have conferred on the organisms the ability to utilize, conceivably derive their nutrients from the compounds and grow in the presence of hydrocarbon [41,42]. [43] has reported that *Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB and *Bacillus subtilis* 106PB showed high lipase activity on solid media indicating their ability for degrading lipid (oil) as carbon source and producing lipase enzyme. From Table 4, microbial count after the treatment T1 (10g treatment), ranged from 2.5×10^6 – 4.5×10^5 , after treatment T2 (50g treatment) microbial load ranged from 1.4×10^5 – 2.5×10^5 and after treatment T3 (100g treatment) microbial load ranged from 1.2×10^5 – 2.0×10^4 . It was observed that the higher the weight of adsorbents the better the removal efficiency resulting in a decrease in the microbial population. Other studies reported that combined carbons from agro-wastes offered better attachment pores for contaminants [43,44]. [43] reported that agricultural waste-based activated carbon contains antimicrobial activity against pathogenic *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In a related work, activated carbon derived from rice husk and coconut husk was found to be effective in decontaminating water containing *Escherichia coli*, by attaching to activated carbon through strong LiftShitz and vanderWaals forces, the carbons showed > 99% removal of *E. coli* [45]. Further examination using SEM (Scanning Electron Microscope) and BET (Brunauer Emmett Teller, particle surface area measurements) results reveal that the carbons were mesoporous in nature while FTIR (Fourier-transform infrared spectroscopy) showed the presence of functional groups viz. C = O and -OH that might be responsible for adsorption of *E. coli* on the carbon [46].

Morphological and Biochemical test as presented in Table 5. Functional microorganism identification includes *Staphylococcus aureus*, *Bacillus subtilis*, *Clostridium botulinum*, *Pseudomonas aeruginosa*, *Proteus vulgaris*,

Escherichia coli, *Klebsiella pneumonia* are the main microorganisms in petrochemical wastewater after treatment with activated rice husk.

Bacterial isolates from both untreated and treated effluent were identified based on their morphological, Gram reaction and biochemical reactions. Standard tests include; Gram reaction and shape, catalase which demonstrates the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide; motility which indicated diffused growth from the straight line of inoculation during incubated at 37°C for 24 hours indicates positive result for motility, bile solubility test, aerobic growth, anaerobic growth, coagulase which identifies whether an organism produces exoenzyme. This enzyme clots the blood plasma by a mechanism that is similar to normal clotting. Coagulase is an enzyme that clots blood plasma. Coagulase is a virulence factor of *S. aureus*. The formation of clot around an infection caused by these bacteria likely protects it from phagocytosis. This test differentiates *Staphylococcus aureus* from other coagulase negative *Staphylococcus* species. Oxidase test checks the presence of terminal enzyme cytochrome c oxidase or cytochrome a, [47]. Cytochrome oxidase transfers electrons from the electron transport chain to oxygen (the final electron acceptor) and reduces it to water. Members of entrobacteriaceae give negative oxidase test, Blue-purple colour indicated the presence of terminal enzyme cytochrome c oxidase or cytochrome a. Sulfur can be reduced to H₂S (hydrogen sulfide) either by catabolism of the amino acid cysteine by the enzyme cysteine desulfurase or by reduction of thiosulfate in anaerobic respiration. If hydrogen sulfide is produced, a. *Proteus mirabilis* is positive for H₂S production, Decarboxylase, Citrate utilization, urease test identifies bacteria capable of hydrolyzing urea using the enzyme urease. It is commonly used to distinguish the genus *Proteus* from other enteric bacteria. The hydrolysis of urea forms the weak base, ammonia, as one of its products. Amino acids are metabolized variably by gram negative aerobic and facultatively anaerobic bacteria as well as gram positive cocci. These amino acids are decarboxylated, hydrolysed or deaminated depending on the organism and the amino acid in question. In decarboxylation, the enzymes break the bond holding the carboxylic (-COOH) group to the rest of the amino acid. The phenylalanine serves as the substrate for enzymes, which are able to deaminate it to form phenylpyruvic acid. Yeast extract in the medium supports the growth of the organisms. Sodium chloride maintains osmotic equilibrium.

Table 4. Identified bacterial isolates of effluent before treatment and after treatment (Pre- treatment) (cfu/ml)

Bacterial isolates	UT	T1	T2	T3
Gram+ve				
<i>Staphylococcus aureus</i>	6.4x10 ⁶	2.5x10 ⁶	2.2x10 ⁶	2.0x10 ⁴
<i>Bacillus subtilis</i>	4.9x10 ⁶	2.9x10 ⁶	2.1x10 ⁴	1.8x10 ⁶
<i>Clostridium botulinum</i>	5.9x10 ⁴	2.4x10 ⁵	1.4x10 ⁵	1.2x10 ⁵
Gram-ve				
<i>Pseudomonas aeruginosa</i>	6.9x10 ⁴	3.5x10 ⁴	1.5x10 ⁴	1.4x10 ⁶
<i>Proteus vulgaris</i>	6.5x10 ⁶	4.5x10 ⁵	2.5x10 ⁵	1.3x10 ⁴
<i>Escherichia coli</i>	5.5x10 ⁵	2.4x10 ⁶	2.3x10 ⁶	1.5x10 ⁴

Key: UT= Untreated Effluent, T1= Treated Effluent 1 (10g treatment), T2= Treated Effluent 2 (50g treatment), T3= Treated Effluent 3 (100g treatment).

Table 5. Morphological and Biochemical test

Morphological and Biochemical test	Isolates						
Gram reaction and Shape	+ve/cocci	+ve/rod	+ve/rod	-ve/rod	-ve/rod	-ve /rod	-ve /rod
Catalase test	+ve	+ve	-ve	+ve	+ve	+ ve	+ve
Motility	-ve	+ve	+ve	-ve	+ve	+ ve	-ve
Bile solubility test	+ve	-ve	-ve	-ve	- ve	- ve	-ve
Aerobic growth	+ve	+ve	-ve	+ve	+ ve	+ ve	+ve
Anaerobic growth	-ve	-ve	+ve	-ve	- ve	- ve	-ve
Coagulase test	+ve	-ve	-ve	-ve	- ve	- ve	-ve
Oxidase test	-ve	-ve	-ve	+ve	+ ve	- ve	-ve
Hydrogen sulphide Production	-ve	-ve	+ve	-ve	- ve	- ve	-ve
Decarboxylase test	-ve	-ve Ornithine	+ve Arginin	+v Arginin	- ve	+ve lysine	-ve Ornithine
Citrate utilization test	-ve	+ve	-ve	+ve	- ve	+ ve	+ve
Glucose fermentation Test	+ve	+ve	+ve	-ve	- ve	+ ve	+ve
Sucrose fermentation Test	+ve	+ve	-ve	-ve	- ve	+ ve	+ve
Lactose fermentation Test	+ve	+ve	-ve	-ve	+ ve	+ ve	+ve
Urease test	+ve	-ve	+ve	-ve	+ ve	+ ve	+ve
Indole test	-ve	-ve	-ve	-ve	+ ve	+ ve	-ve
Methyl red	+ve	-ve	-ve	-ve	- ve	+ ve	-ve
Deoxyribonuclease	+ve	-ve	+ve	-ve	-ve	- ve	-ve
Phenylalanine Deaminase	-ve	-ve	+ve	-ve	+ ve	- ve	-ve
	<i>S.aureus</i>	<i>B.subtilis</i>	<i>C.butilinum</i>	<i>P.aeruginosa</i>	<i>P.vulgaris</i>	<i>E.coli</i>	<i>K.pneumoniae</i>

Key; *S.aureus*= *Staphylococcus aureus*, *B.subtilis*= *Bacillus subtilis*, *C.butilinum*= *Clostridium butilinum*, *P.aeruginosa*= *Pseudomonas aeruginosa*, *P.vulgaris*= *Proteus vulgaris*, *E.coli*= *Escherichia coli*, *K.pneumoniae*= *Klebsiella pneumonia*.

Microorganisms that produce phenylalanine deaminase remove the amine (NH₂) from phenylalanine. The reaction results in the production of ammonia (NH₃) and phenylpyruvic acid [47]. The ability of certain bacteria to decompose the amino acid- Tryptophan to Indole, methyl red determine which fermentation pathway is used to utilize glucose. In the mixed acid fermentation pathway, glucose is fermented and produces several organic acids (lactic, acetic, succinic, and formic acids). The stable production of enough acid to overcome the phosphate buffer will result in a pH of below 4.4, and fermentation of the following sugars: glucose, fructose, lactose) on the isolates to identify bacteria associated with the untreated effluent, and treated effluent [48].

Nevertheless, the treatment process depleted microbial growth, the better performance of rice husks was probably due to its chemical composition, which favored reduction of microbial growth. The lack of nitrogen source and excessive lignin content of the other tested biomasses may have hindered microbial growth, causing nutrient deficiency and formation of inhibitor by-products due to lignin degradation, respectively [6,48].

This work provides new insights into the microbial ecosystem in the petrochemical wastewater treatment process. Biological wastewater treatment plants based in activated rice husk have been in charge of treating both domestic and industrial wastes and have constituted an essential instrument in environmental protection due to the removal of organic matter, suspended soils, nutrients (N, P) and pathogens [13].

4. Conclusion and Recommendation

Rice husk, an agricultural by-product, acts as an effective adsorbent for the removal of pollutants from oily effluent. Its application will act as a contribution to adopt eco-friendly approaches in the treatment of effluents. Thus, the adsorbents can provide alternative, cost effective, non-chemical and less toxic way of treating wastewater with high microbial load. This findings may hereby establish the adsorptive nature of rice husk and its efficacy and also in the treatment of effluents from oil industries. It is hereby recommended that in the treatment of petroleum

effluent with a view of reducing the microbial population and physicochemical properties rice husk is effective and recommendable before being discharged into the water bodies.

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Statement of Competing Interests

The authors declared that there is no conflict of interest.

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