Original Research Article

AEROBIC BIOREMEDIATION OF BREWERY EFFLUENT USING *Escherichia coli* and *Pseudomonas sp.*

ABSTRACT

Effluent from Guinness Nig. Plc was obtained and inoculated with two aerobic organisms, and physicochemical analysis was carried out to evaluate the intrinsic degradative capabilities of the organisms based on the parameters to determine which organism has a better degradative potential for the effluent. This study used two bacterial species, *Escherichia coli* and *Pseudomonas sp.* The study was divided into two setups for each microorganism, and the third was set up as a consortium of the two. The study showed a reduction in biological oxygen demand (BOD) and a simultaneous reduction in the inorganic nutrient content of the effluent, such as phosphate, nitrate and sulphate, which were believed to be utilized for the growth of their biomass.

Keywords: *Aerobic, Bioremediation, Brewery, Escherichia coli and Pseudomonas spp*

1.0 INTRODUCTION

Effluents are wastewater and could be defined as water that environmental conditions have transformed to such an extent that it becomes unsuitable for direct use and domestic purposes such as drinking water, dishwashing, laundry, bathing and general sanitary conditions [1]. It can also be defined as liquid or water waste discharged from an industrial establishment [2]. According to Rodrigues et al. [3], typical wastewater might include unconsumed inorganic media components, microbial cells and other suspended solids: filters and waste wash water from cleaning operations, cooling waters and water containing traces of solvents, acids, alkalis and human sewage. Effluent is the liquid discharged from a processing step [2].

Industrial effluents vary in load, concentration of pollutants, and toxic material. They are often nutritionally unbalanced, they may contain biodegradable or non-biodegradable or both types of pollutants, and they show seasonal variations in their relations production [4]. There are different industrial effluents categories, including pharmaceutical, chemical and petrochemical, coal conversion, food and dairy processing, and brewery effluents.

Brewery effluent is wastewater from a brewery [4]. Brewery wastes are composed mainly of liquor pressed from wet grain and liquor from yeast recovery and wash water from the various departments [5]. Brewery wastes contain an average of 72ppm of suspended solids and 390ppm of BOD. when 6996

bushels of barley are processed per day. It contains 99.9% of water, the remaining 0.1%, i.e. 1g/l, is made of approximately 70% organic and 30% inorganic solids. Half of the solid contents are suspended and half dissolved [6].

There are two main categories of biological treatment: aerobic treatment or treatment in the presence of oxygen or air and anaerobic treatment or treatment in the absence of oxygen. Some crossover processes utilize limited oxygen or air and are known as Facultative or anoxic processes [5]. The ultimate goal of biological treatment is to reduce or mineralize organic compounds into carbon dioxide and water. The degradation of organic compounds by biological organisms is called Biodegradation [7, 8, 9, 10]. One of the newest and fastest-growing applications of environmental biotechnology is bioremediation. Bioremediation uses microorganisms to degrade environmental pollutants from the environment, thus restoring the original natural surroundings [11].

It is a technique that enhances the natural rate of biodegradation of pollutants through the activities of degradative microorganisms. Bioremediation is an attempt to facilitate the restoration rate of waste upon pollution with recalcitrant molecules [5]. Bioremediation of organic waste is becoming an increasingly important waste treatment method [12. 13, 14, 15].

2.0 MATERIALS AND METHODS

2.1 Sources and Sample Collection

The microorganism used for the work included *Escherichia coli* and *Pseudomonas spp* and were procured from the Federal Institute of Industrial Research, Oshodi (FIIRO). The effluent was collected from Guinness Plc Nigeria Ikeja, Lagos, Nigeria.

2.2 INOCULATION OF NUTRIENT BROTH WITH MICROORGANISMS

Four calibrated 500mls sterile conical flasks with 2 containing 250mls of prepared nutrient broth were labelled *Escherichia coli* broth as E_1, *Pseudomonas spp* as P_1 and the other two containing 125ml of broth and labelled *Escherichia coli* as $E_2$and *Pseudomonas spp* as P_2. Six previously inoculated nutrient agar plates were used; three plates were inoculated with E. coli while the other three were with *Pseudomonas spp*. 2mls of uninoculated broth was then injected into the plates, and a sterile inoculated loop was used to scrape the surface of the plates; the colonies formed into the respective labelled conical flask and then incubated at 37^0C for 24 hours.

2.3 MEASUREMENT OF MICROBIAL GROWTH

2.3.1. Turbidity reading

Turbidity is a measure of the light-transmitting properties of a liquid [2]. The turbidity readings of the inoculated broth were taken twice daily at an interval of 6 hours using a spectrophotometer at 620nm. Aseptic conditions were practised when taking the readings using a Bunsen burner, and cotton moistened with alcohol was used to swab the bench and the sides of the cuvettes used. Measurement of microbial mass is determined by taking the turbidity reading based on light absorption.

2.3.2 Standard Plate Count (SPC)

Microbial growth was measured by counting the number of colonies displayed on plates. Spread plate technique was used for isolation. In the spread plate technique, 0.1ml of inoculated broth was aseptically transferred into Petri dishes' solid nutrient agar medium. Then a sterile hockey slick was used to spread the inoculums evenly to cover the entire agar surface. The spreading of the inoculums was carried out under aseptic conditions to avoid contamination. The plates were incubated at 37^0C for 24 hours. The colonies which appear were counted.

2.4 INOCULATION OF EFFLUENT WITH BROTH

Four clean, sterile plastic containers were labelled

E: Effluent and broth inoculated with *Escherichia coli*

P: Effluent and broth inoculated with *Pseudomonas* sp.

M: Effluent and broth inoculated with *Escherichia coli* and *Pseudomonas* sp.

E: Effluent alone (uninoculated).

2 litres of fresh untreated effluent were measured using a measuring cylinder and poured into each bowl. The inoculated nutrient broth contained in the conical flask was poured into the bowls described by manufacturers. The 4 setups (samples) were left uncovered and mixed frequently using a glass rod labelled appropriately for each placed in a confined area in the laboratory for aeration

2.5 MICROBIOLOGICAL ANALYSIS OF THE SAMPLES

The media used for this analysis was (PDA) to isolate fungi and nutrient agar to isolate bacteria. Method of application for isolation was the spread plate technique.

2.5.1 Serial Dilution Techniques (SDT)

Serial Dilution Techniques were applied to thin out high population densities of microorganisms present in the samples to a countable number [16, 17]. The SDT used was the ten-fold serial dilution. The diluents (distilled water) were sterilized in the autoclave at 121^0C for 15 minutes. The diluents were allowed to cool to room temperature before use so that if do not denature the microorganisms present in the effluent. Ten test tubes containing 9mls of sterile diluents were arranged in a clean test tube rack labelled 1-10. 0.1ml was taken from the effluent using a syringe (needle) calibrated 2mls and delivered into test tube 1 to give 10^{-1} dilution. The sample was thoroughly mixed so that the quantity added is a representative sample of the bulk sample to be analyzed. Then from tube 1, 1ml was aseptically transferred to tube 2 to give 10^{-2} dilution. This procedure was repeated subsequently with tube 10 to give10^{10}. The dilution factor for each sample was 10^{-3}, 10^{-6}, 10^{-9}.

2.6. DETERMINATION OF MICROBIOLOGICAL AND PHYSICO-CHEMICAL PARAMETERS OF SAMPLES.

2.6.1 Microbiological analysis

2.6.1.1. Total bacteria (viable) count

The total bacterial counts of the samples were obtained by aseptically introducing aliquots (0.1ml) of appropriate dilution on a sterile nutrient agar plate by spread plate techniques. The plates were incubated at 37^0C for 18-24 hours. The total viable count of the samples was estimated based on the colony counts and multiplied by the reciprocal of the dilution factor and reported as a colony-forming unit.

2.6.2. Fungal population

The total fungal count of the samples was obtained by aseptically introducing 0.1ml of the appropriate dilution on PDA by spread plate techniques. The PDA medium was supplemented with streptomycin (100μg/ml) to inhibit the growth of bacteria and incubated at 30^0C for 48-72 hours. The fungal population were estimated as described above and reported as colony-forming units.

2.7. DETERMINATION OF PHYSICOCHEMICAL PARAMETERS

2.7.1. temperature

The temperature of the effluent sample was determined using the mercury bulb thermometer, and the thermometer was dipped into the effluent sample and left for 3 minutes. Readings were determined from the rise in the level of mercury.

2.7.2. Hydrogen ion concentration (pH)

The pH of the wastewater samples was determined using a portable pH meter with combined glass and calomel electrodes. The pH meter was standardized with buffer solution pH 4.0, 9.2 and 7.0 after which the samples were tested in turn.

2.7.3. Appearance and Odour

The appearance and odour of the samples were determined by an organoleptic method using physical observation and by smelling the samples.

2.7.4. Sulphate (SO_4^{2-}), Nitrate (NO_3^-) and Phosphate (PO_4^{3-})

The SO_4^{2-}, NO_3^- and PO_4^{3-} were determined using the HACH DR 2000 direct reading spectrophotometer, which is internally calibrated. For each parameter, the HACH reagents in the pillow were used and added to 25ml of the effluent sample, and after the reaction time, the concentration was read directly in the spectrophotometer after zeroing with the blank. Each parametersSO_4^{2-}, NO_3^-, and PO_4^{3-}has their respective wavelength of best absorption, SO_4^{2-} (450nm), NO_3^- (500nm) and PO_4^{3-} (890nm).

2.7.5. Biological Oxygen Demand (BOD_5^{20})

The BOD_5^{20} was carried out by determining the dissolved oxygen content of the sample at zero-day, day of sampling and fifth day after incubation at 20^0C in a dark bottle. The difference between dissolved oxygen at zero-day and that of fifth, multiplied by the dilution factor, gives the BOD_5^{20}.

2.7.6. Chemical Oxygen Demand (COD)

The COD was determined by first oxidizing some quantity of the effluent sample with potassium dichromate in the presence of a catalyst and the excess titrated with ferrous ammonium sulphate using ferroin as an indicator.

2.7.7. Total Dissolved Solids (TDS)

The effluent sample's total dissolved solids were determined using the conductivity meter (E587 model in Switzerland). The conductivity of the samples was read from the conducting meter at 0.666 for total dissolved solids then the results were multiplied.

3.0 RESULTS

Changes in Physiochemical and Microbiological Parameters

The growth profiles of the selected bacteria *Escherichia coli* as E_1, *Pseudomonas spp* as P_1 and *Escherichia coli* as E_2 and *Pseudomonas spp* as P_2 were grown in Nutrient Broth (NB). The nutrient broth supported the remarkable growth of the organisms. Thus, the starters inoculated in the effluent were all in their exponential growth phases, as reflected by the optical density (OD) values. The bacteria cultures of E_1, P_1, E_2, P_2, which were at an exponential growth phase, contained 3.82 x 109, 3.52 x 109, 2.37 x 109 and 2.54 x 109cfu/g, respectively, inoculated in the experimental setups. E, P, M, respectively, with sterile distilled water into the control setup. Table 1 shows the mean changes of the physicochemical and microbiological parameters in the experimental setup and the control. This shows that the inorganic nutrient concentration such as sulphate, phosphate and nitrate levels decreased more rapidly with the proportional increase in the population densities of *E. coli and Pseudomonas sp, a*nd the consortium of both in setup E, P, and M, respectively. In the Experimental setup, E, P, M $\log_{10}$ transformed data of colony-forming units (cfu/g) obtained for bacteria showed significant decreases in the BOD level. However, between weeks 6-10, the BOD level reduction trend was not as rapid as between weeks 1 and 3 and the changes in the microbial population. In the control setup, the trends of the results were significantly slow and relatively inefficient, particularly at the BOD level.

Table 1: Mean changes in physicochemical and microbiological analysis.

Wks	Sample	pH	SO_4^- [2]mg/l	NO_3^- [1]mg/l	PO_4^- [3]mg/l	COD mg/l	BOD mg/l	TDS mg/l	Microbial Population	Mean
0	E	8.60	20	48	43	2401	1201	70.0	5.1×10^9	
	P	8.65	22	50	44	2622	1311	76.2	4.2×10^8	
	M	8.51	18	40	41	2200	1100	60.0	6.3×10^9	
	C	8.60	24	50	45	2401	1201	78.2	2.7×10^3	
2	E	8.42	18	37	40	2000	1000	35.0	5.9×10^9	
	P	8.51	19	45	42	2310	1155	42.0	4.7×10^8	
	M	8.30	16.2	31	37	1800	900	31.0	6.9×10^9	
	C	8.62	23.2	49	44	2400	1200	65.0	3.0×10^4	
4	E	8.40	15	30	39	1800	900	23.0	6.5×10^9	
	P	8.48	17	40	40	1900	950	31.5	5.1×10^9	
	M	8.30	13.1	25	27	1450	725	20.0	7.3×10^8	
	C	8.60	22.1	48.5	41	2300	1150	59.0	3.6×10^5	
6	E	8.36	15	28	38	1200	600	15.0	7.1×10^9	
	P	8.40	17.2	32	40	1350	675	22.0	6.4×10^9	
	M	8.42	12.2	23	25	800	400	14.6	7.8×10^8	
	C	8.40	21.9	48.1	40	2150	1075	57.9	3.9×10^6	
8	E	8.10	12	22	30	800	400	8.5	7.6×10^9	
	P	8.32	15	25	35	900	450	11.5	6.7×10^9	
	M	7.80	11.7	18	21	320	160	8.0	7.3×10^8	
	C	8.10	21.5	47.9	40	2100	1050	40.7	3.5×10^7	
10	E	7.80	9	15	20	650	325	5.0	4.5×10^{11}	
	P	7.90	13.5	18	25	720	360	7.5	4.0×10^{11}	
	M	7.30	8.7	10	17	180	90	4.6	5.1×10^{10}	
	C	7.90	21.1	47.5	39.1	200	1000	38.6	2.5×10^3	
12	E	7.61	8	9	12	310	155	1.5	3.4×10^{11}	
	P	7.52	12	2	18	350	175	3.0	3.0×10^{11}	
	M	7.1	7.5	9	10	150	75	1.1	4.2×10^{10}	
	C	8.20	21	47.0	39.0	1800	700	30.2	2.1×10^4	

4.0 DISCUSSION

The bioremediation of brewery effluent was successful using both pure strains of microorganism, *Escherichia coli* and *Pseudomonas spp* and the consortium of both bacteria due to the environment provided by the effluent sample.

A noticeable change in colour from the original dirty brown colour to a lighter brown colour of the sample was the first pointer to the fact that bioremediation was suspected of having taken place. The effluent was turbid and cloudy at first on observation and later transformed to a more transparent liquid. This emitted a noxious odour during the bioremediation process, which established that the organism utilized the organic material present in the effluent, leading to the production of various gases such as methane, butane, and ammonia. Our findings were in tandem with the findings of [3], who found an odour from the effluent during the bioremediation process.

The results from this study also show that *Escherichia coli* and *Pseudomonas spp* have almost equal capabilities of degrading brewery effluent, with *Escherichia coli* showing a higher efficiency which was in agreement with the findings of [18], who found that effluent from dairy technology of high efficiency of some microorganisms. Another important observation of this study was that bioremediation could proceed equally well when a mixed culture of microorganisms was used. *Escherichia coli* and *Pseudomonas spp* have various enzymes that are important in cleaning the environment polluted by organic compounds. They can be said to be microbes that possess broad-spectrum bioremediating capabilities because they have enormous catabolic potentials, which confirms the findings of Das and Santra, [19].

The environmental conditions that determine the organism's growth are temperature, aeration, nutrient availability inorganic, nitrogen, phosphate, sulphate, trace elements, oxygen concentration, and pH and substrate concentration (organic) [20, 21]. The effluent setup of E, P, C, M was mixed frequently, which allowed for aeration, enabling the substrate present in the effluent to be available for the microorganism, which points to the fact that the more intense the mixing, the more substrate will pass by the microorganism and the faster the microorganism can utilize the substrate. Based on the aeration, the higher the oxygen concentration, the faster the occurrence of metabolic reaction if no nutrient is limited.

Metabolic reactions occur fastest at optimum pH, usually defined as 6-8, but there are rare instances of maximum rate reactions occurring outside this range [22]. The pH results from the analyses were stable

during the bioremediation process and between the ranges of 6-8 and increased slightly over the period in all the setup, which suggests that alkaline substances were produced as a result of degradation of the effluent. Microorganisms require nutrients such as nitrogen, phosphorus and trace elements to grow, and lack of these nutrients slows or stops growth prematurely. The nutrients are gotten from the raw material used during production, starchy materials such as barley, corn, rye, and wheat. Corn, rice, potatoes and materials containing sugar, such as high sugar molasses, fruits, sugar beets [23], which backed up the result of the analysis shown in the table that there was a sudden increase in the microbial population in the first 0-6 weeks and reflected a gradual loss of inorganic nutrients most particularly the phosphate PO_4^{3-}, Nitrate NO^{3-} and Sulphate SO_4^{2-} in the experimental setup E, P, M, then in the control chamber C which was as a result of the metabolism of the microbial population. For example, the consistent decrease in PO_4^{3-}, may be due to its high demand by microorganisms for sugar phosphorylation as a component of cell membranes (phospholipids) and nuclear acids (nucleotides). Microorganisms exploit nitrate NO_3^{-1} and SO_4^{2-} sources to meet their protein and nucleic acids requirement [22]. Utilization rates of these nutrients are normally proportional to the concentration of a growth-limiting substrate. The aeration and agitation ensured that the biomass was maintained in a suspended state to maximize oxygen and nutrients, as earlier workers reported [24].

The temperature profile was stable over the period in all the setup and adapted to the temperature of the environment in the laboratory, room temperature.

5.0 CONCLUSION

Based on the studies carried out, both *Escherichia coli* and *Pseudomonas spp.* Possess bioremediation capabilities, and therefore the difference between the result observed from the physicochemical and biological analysis for the experimental setup E, P, M, and control setup C in terms of efficiency of the bioremediation process could be attributed to the positive contributions made by manipulating the environmental parameters. In this study, the environmental parameters manipulated are the addition of beneficial starters of *Escherichia coli, Pseudomonas spp* and aeration and agitation of the setup, thus the significant changes in the measured physiochemical variables.

Ethical approval and consent to participate

Not applicable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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