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Original Research Article

The microbiological and physicochemical characterization of wastewater from a brewery in southwest Nigeria: A case study

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The study assessed the microbiological and physicochemical parameters of wastewater collected between September 2008 and August 2009 from the sedimentation tank inlet and the discharge point of the oxidation ponds of a brewery with a view to determine the efficiency of the wastewater treatment system. The influent wastewater had mean values of total bacterial, coliform and fungal densities of 9.8 × 10^5 cfu/mL, 4.7×10^2 cfu/l00mL, and 2.8 × 10^4 cfu/mL respectively while the discharged effluent had mean values of 2.9×10^6 cfu/mL, 9.0 × 10^3 cfu/l00mL and l.5 x 10^5 cfu/mL respectively. The DOM, BOD and TS of effluent had mean values of 26.0 mg/L, 210.0 mg/L and 406.6 mg/L, respectively, while the mean of the DO concentration was 0.9 mg/L. The mean of the pH and temperature of the influent wastewater were 10.0 ± 0.02 and 38°C ± 0.5, respectively, while those of the discharged effluent were 7.3 and 35°C respectively. The concentrations of mercury and lead in the discharged effluent were higher than acceptable limits.The findings showed that the efficiency of the oxidation ponds was low and hence poses a potential danger to the receiving environment.

Key words: Brewery effluents, coliform, oxidation ponds, wastewater

INTRODUCTION

Industrial wastewaters are wastewater generated from industries and associated processes that utilize water (Alebel, 2010). These kinds of wastewater are mixtures of various suspended solids, nutrients, biodegradable organics, pathogens, heavy metals, refractory organics, and dissolved inorganic solids. The relative compositions vary widely depending on the type of activity producing the wastewater. The amount and toxicity of waste released by an industry is also directly related to its own specific activity (Nkonyeasua, 2010). Most industries discharge their wastewaters into the surrounding environment especially in bodies of water located close to such industries. Such discharges could impact the ecology of the receiving environment if not pretreated at the source point and may result in discoloration, foul smells and death of aquatic life, apart from making the water unfit for various other purposes. Industrial wastewaters, therefore, pose a significant pollution threat to the environment, receiving water-bodies and soil, hence the quality of the effluent must be controlled (WHO, 2006).

Wastewater treatment is the most important way of ensuring that it is properly handled before discharge into the environment. There are a number of methods through which wastewater treatment can be carried out. However, wastewater treatment through the use of waste stabilization ponds (WSP) has been considered as an ideal way of improving effluent quality by means of natural processes. Waste stabilization ponds, also known as oxidation ponds, are considered as an effective, inexpensive, and simple technology useful in wastewater treatment system in developing countries (Von Sperling and De Lemos, 2005). They are usually the most preferred systems in hot climatic zones and are very effective at removing pathogens (Punmia et al., 2005). The treatment of wastewater occurs through natural physical, chemical, or biological processes at no extra energy requirement except the sun and is capable of providing a very high-quality effluent (Argaw, 2003).

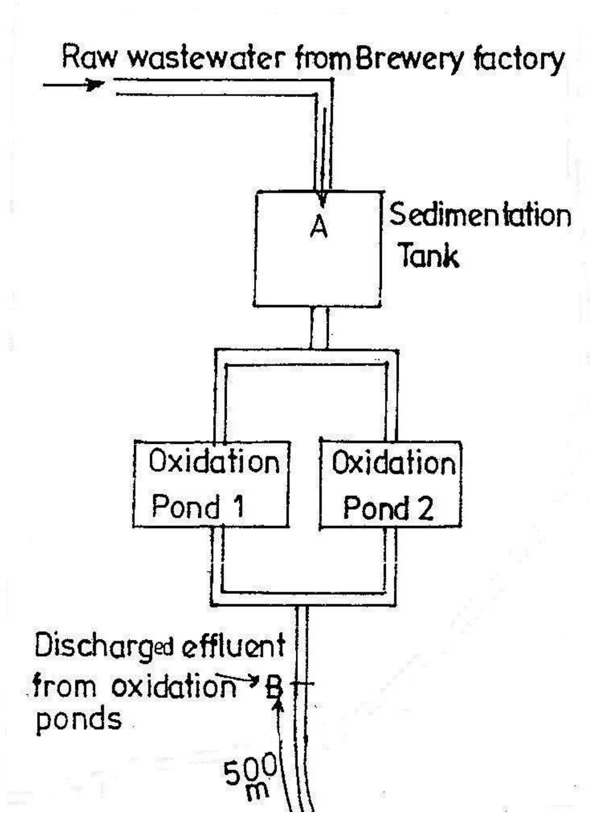

Figure 1: Scheme of the sampling stations (not drawn to scale).

The oxidation ponds constructed by the International Breweries Plc has been used for the treatment of wastewater since 1993. This study was carried out to determine the biological and physico-chemical parameters of the brewery wastewater before and after treatment in the oxidation ponds.

MATERIALS AND METHODS

Study area

The study was carried out on the oxidation ponds system constructed in the premises of International Breweries Plc. located in Ilesa town, Osun state, Nigeria. The brewery occupies an area of about 4,582 square meters and produces beer and malt drinks. The wastewater from the factory enters directly into a sedimentation tank through gated entrance (sampling point A) (Figure 1). Each oxidation pond is rectangular in shape and cross section with length 19.202 m, breadth 14.630 m and height 1.829 m. Each pond is equipped with a V-notch weir for outflow of the treated water out of the oxidation pond at $60m^3/h$ before the effluent is discharged out of the company's premises (sampling point B). Samples were collected at each location once every two months from September 2008 to August 2009.

Collection of water samples

Samples were collected at about 10 cm below the water surface into 2 L kegs pre-disinfected with 75% ethanol and rinsed with sterile distilled water. Water from each sampling point was used to rinse the appropriately labeled keg twice before sample collection. For the microbiological analysis, samples were transported to the laboratory on ice and analyzed within three hours of collection.

Microbial analysis of water samples

Total microbial counts

The enumeration of total microbial count was carried out using the serial dilution and pour plate technique. Serial 10 fold dilutions in sterile water were carried out and 1mL of each dilution was aseptically placed in sterile Petri dishes in duplicates. Twenty mL of molten nutrient agar (Oxoid) cooled to 45°C for bacterial population or Potato Dextrose Agar (Oxoid) for fungi was later added to each of the plates. The mixture was allowed to solidify and incubated appropriately for 24 hours for bacterial and 7 days for fungal populations.

Presumptive coliform count

The water samples were processed using the multiple fermentation tube method to determine the Presumptive coliform count/most probable number (MPN) of coliforms based on standard methods (Senior, 2006). Suspensions from positive tubes were subcultured onto MacConkey agar and incubated at 37°C for 24-48 hours. The resulting colonies were identified following standard procedures (Forbes et al., 2007).

Detection and enumeration of faecal coliforms

The Eijkman's test involving the use of elevated temperature for the differentiation of faecal and non-faecal coliforms was employed. Measured volume of water samples were inoculated into lactose broth in similar combinations as in the presumptive test for total coliforms and incubated at 44 °C for 48 hours. The number of positive tubes was recorded at the end of the incubation period and the MPN of faecal coliform was read from the MPN index table.

Isolation of pure cultures of microorganism

Distinct colonies from the mixed culture on each of the nutrient agar and the potato dextrose agar plates were picked and streaked onto fresh plates. Pure colonies of each bacteria and fungi were then picked for identification using

Table 1: Microbiological parameters of the brewery wastewater and the effluent

Period	Sampling points	Total Bacteria (cfu/mL)	Coliform (cfu/100mL)	Faecal coliform (cfu/100mL)	Fungi (cfu/mL)
Sept	A	3.40×10^3	4.20×10^2	0	2.20×10^3
2008	B	3.50×10^4	1.50×10^4	0	8.00×10^3
Nov	A	5.10×10^4	9.00×10^2	0	2.80×10^3
2008	B	8.00×10^5	1.30×10^4	0	1.70×10^4
Feb	A	2.30×10^4	2.50×10^3	0	2.80×10^3
2009	B	9.50×10^5	1.80×10^4	0	5.60×10^3
Apr	A	1.80×10^6	2.30×10^2	0	9.00×10^4
2009	B	1.10×10^7	3.60×10^3	0	4.40×10^5
Jun	A	1.90×10^6	1.90×10^2	0	7.00×10^4
2009	B	7.20×10^8	4.30×10^2	0	2.00×10^5
Aug	A	2.10×10^5	2.50×10^2	0	2.70×10^3
2009	B	2.90×10^6	1.60×10^3	0	2.40×10^5

KEY: A: Brewery raw wastewater; B: Brewery discharged effluent

standard biochemical tests which included Gram's stain, spore stains, catalase, MRVP, citrate utilization, indole, urease and hydrogen sulphide production tests for bacteria and sugar fermentation tests for fungi (Forbes et al., 2007).

Physico-chemical analysis

Standard methods for the examination of water and waste waters (APHA 2001) were used in the determination of the physicochemical parameters of the water samples. Colour, temperature, pH, and Dissolved Oxygen were determined on site.

The phosphate content was determined using phosphate Test Kit (model PO-23A). The nitrate content in the water samples was determined using Nitrate Test Kit of model N1-11. Iron Test Kit Model IR-18 was used for the determination of iron content while the chlorine content of the water samples was determined using Free and Total Chlorine Test Kit Model CN-70.

The concentrations of heavy metals (Mercury, Arsenic, Lead, Chromium and Cadmium**)** in the water samples were determined using Atomic Absorption Spectrophotometer (AAS) at the Central Science Laboratory of Obafemi Awolowo University, Ile-Ife and also at the Centre for Energy Research and Development, (CERD), O.A.U., Ile-Ife.

Statistical analysis

Data obtained from the study was analyzed using Microsoft Excel package.

RESULTS

The microbiological parameters which included bacterial, fungal, coliform and faecal coliform counts are presented in Table 1.

Dominant genera of organisms isolated from the sampling points

A total of 16 strains of bacteria representing 8 genera and 9 species of fungi were isolated from the factory raw wastewater and the treated effluent as shown in Table 2. Six fungal species which included *Trichophyton* spp., *Microsoprium* spp., *Scepulariosis* spp., *Saccharomyces* spp. and *Aspergillus* spp. were isolated from the raw brewery wastewater from the factory while 3 additional species were isolated from the discharged effluent.

Presented in Table 3 are the biochemical and physical parameters of the influent wastewater and the effluent while Table 4 shows the chemical parameters of the influent wastewater and the effluent.

DISCUSSION

As shown in the results obtained in this study, the brewery effluent discharged from the oxidation ponds contained more bacterial loads than the influent wastewater and the coliform count exceeded the limits set by the National Environmental Standards and Regulations Enforcement Agency (NESREA) for industrial effluent discharge into surface waters. In oxidation ponds, pathogens are expected to be progressively removed along the ponds series with the highest removal efficiency taking place in the maturation ponds. Several factors have been reported to determine the rate of removal of bacteria in oxidation ponds and the bacterial load in the final effluent. These include the size and number of the maturation ponds, the BOD and intensity of sunlight (Sinton et al., 2002; Ainon and Chuan, 2002).

The bacterial population of the raw brewery wastewater and discharged effluent was observed to vary with season and the changes in the composition of wastewaters due to

Table 2. Organisms isolated from the sampling points

Organisms	Sampling points	
	A	B
Bacteria		
Actinomyces species	-	+
Bacillus species	+	+
Corynbacterium species	-	+
Enterobacter species	-	+
Klebsiella species	+	+
Listeria species	+	+
Pseudomonas species	+	+
Streptomyces species	+	+
Fungi		
Absichia spp.	-	+
Aspergillus spp.	+	+
Cladosporium spp.	+	+
Microsoprium spp.	+	+
Mucor spp.	-	+
Saccharomyces spp.	+	+
Trichophyton spp.	+	+
Rhisopus spp.	-	+
Scepulariosis spp.	+	+

Table 3. Biochemical and physical parameters of the brewery wastewater and the effluent

Time	Site	T(⁰C)	CL	DO(mg/L)	BOD(mg/L)	DOM(mg/L)	TS(mg/L)	TDS(mg/L)	TSS(mg/L)
Sept	A	37	LG	3.6	20	2.40	460	240	220
2008	B	35	G	1.2	80	7.69	620	360	260
Nov	A	38	LG	3.2	20	24.36	340	180	160
2008	B	36	DG	0.8	240	52.40	380	200	180
Feb	A	38	LG	2.8	60	17.20	460	300	160
2009	B	37	DG	0.6	220	26.40	520	340	180
Apr	A	39	LG	3.4	40	14.80	180	120	60
2009	B	36	G	1.1	180	21.60	240	200	40
Jun	A	37	G	1.8	80	25.10	340	280	60
2009	B	35	DG	0.6	280	33.60	360	280	80
Aug	A	37	LG	2.4	60	9.60	240	200	40
2009	B	35	G	1.0	260	14.53	320	240	80

KEY: A: Brewery raw wastewater; B: Brewery discharged effluent; T (°C): Temperature; CL: Colour; DO: Dissolved oxygen; BOD: Biochemical oxygen demand; DOM: Dissolved organic matter; TS: Total solids; TDS: Total dissolved solids; TSS: Total suspended solids; LG: Light grey; G: Grey; DG: Dark grey.

the type of product being brewed. The highest bacterial densities in the raw wastewater and in the discharged effluent were recorded in June and this coincided with the production of Betamalt, a non-alcoholic malt drink which changed the composition of the brewery wastewater. Betamalt was brewed and bottled only in this month during the entire period of this study. Malt effluent has been reported to exhibit a higher BOD value than beer effluents and this may lead to an upsurge in bacterial loads and subsequently lower rate of removal of bacteria in the oxidation pond (Ainon and Chuan, 2002). At the peak of the rainy season in the months of August and September, a reduction in the bacterial populations of raw wastewater and the discharged effluents were noticed. This reduction is thought to be related to the high volume of rainfall recorded within the months which fell directly into the oxidation ponds thereby diluting the effluent. The higher rate of evaporation and less rainfall associated with the dry season between November and February are conditions that could favor the concentration of bacteria in the ponds hence causing the gradual increase in the bacterial densities in the raw brewery wastewater and the

Table 4. Chemical parameters of the brewery wastewater and effluent

Period	Point	pH	Concentration in mg/L								
			Nit	Phos	Chl	Iron	Mer	Cad	Chro	Ars	Lead
Sept	A	10.33	4.40	14.00	0.02	0.67	1.20	0.060	0.12	0.72	0.45
2008	B	6.91	12.20	30.00	0.02	2.84	0.88	0.059	0.03	0.68	0.12
Nov.	A	9.90	4.40	28.00	0.02	0.85	1.53	0.086	0.18	0.80	0.55
2008	B	6.10	17.60	40.00	0.02	3.11	0.93	0.088	0.04	0.70	0.16
Feb.	A	9.80	0.00	45.00	0.02	1.63	1.58	0.867	0.27	0.50	0.62
2009	B	6.37	2.00	44.00	0.00	3.04	0.89	0.417	0.07	0.40	0.23
Apr.	A	10.52	1.00	44.00	0.03	0.73	1.46	0.000	0.72	0.00	0.58
2009	B	7.17	1.20	42.00	0.01	2.96	0.84	0.000	0.30	0.00	0.23
June	A	10.37	1.00	22.00	0.02	0.66	1.63	0.920	0.18	0.80	0.56
2009	B	7.08	3.00	12.00	0.00	2.32	0.75	0.066	0.04	0.70	0.18
Aug.	A	10.15	4.40	18.00	0.01	0.72	1.47	0.024	0.27	0.70	0.56
2009	B	6.38	12.20	32.00	0.00	8.48	0.84	0.024	0.21	0.50	0.18

KEY: A: Brewery raw wastewater; B: Brewery discharged effluent Nit: Nitrate; Phos: Phosphate; Chlo: Chlorine; Mer: Mercury; Cad: Cadmium; Chro: Chromium; Ars: Arsenic

discharged effluent. The absence of faecal coliforms in the raw brewery waste and the brewery discharged effluent is an indication that faecal wastes were not being discharged through the same sewer as the brewery effluent. The organisms isolated from the brewery effluent included strains of *Pseudomonas* species and *Corynbacterium* species which are potential pathogens being discharged into the environment.

The dissolved oxygen contents in the discharged effluent were lower than those of the influent wastewater throughout the period of the study. The BOD values of the influent wastewater ranged between 20 and 80 mg/L while the BOD values of the discharged effluent increased drastically to between 80 and 280 mg/L which contravene the stipulated limit of 30 mg/L (Federal Environmental Protection Agency, 1991). The wide difference in the BOD values of influent waste water and the discharged effluent from the oxidation ponds is an indication of the accumulation of dissolved organic matter in the ponds with resultant effect of very low dissolved oxygen and high microbial activities showing that the BOD removal efficiencies of the oxidation ponds were low.

The total solids levels of the influent wastewater and those of discharged effluent were high. The amount of the solid matters in an industrial effluent depends on the type of factory, raw materials used and processing technologies. The high level of total solids are probably due to the presence of spent grains and high dissolved organic matter content of the wastewater from the brewery. The mean value of dissolved solids of the discharged effluent was lower than the stipulated limit of 2000 mg/L (FEPA, 1991), while the suspended solids greatly exceeded stipulated limit. The removal of suspended solids is facilitated by the physical settling of particles and subsequent biological decomposition and mineralization of compounds. The Total Solids removal efficiency of this oxidation pond is therefore below expectation. This may be due to short retention time that does not allow the adequate settling of the suspended solids and the absence of a pre-treatment tank which prevented alum dosing into the raw wastewater which could have helped in the coagulation and sedimentation of suspended and colloidal solids in the brewery wastewater. Also, the sedimentation tank was supposed to be de-sludged periodically to remove accumulated sludge but throughout the duration of this study, no de-sludging took place. The high total solids level in the discharged effluent could lead to a marked increase in the total solids of any receiving streams making them toxic to freshwater animals by causing osmotic stress and disturbing their osmo-regulatory capability (McCulloch et al., 1993). It also renders water unfit for human, industrial or agricultural use.

The pH of the influent wastewater was alkaline throughout the study period ranging from 9.80 to 10.83. This alkalinity is attributable to the use of caustic soda in washing of returned bottles, equipments, floor and tanks. After the detention period in the oxidation ponds, the pH of the discharged brewery effluent dropped to the range between 6.10 and 7.20 which were close to neutral. This is an achievement derived from the use of the oxidation ponds. If the raw wastewater had been discharged without this treatment, the alkaline pH could have undesirable effects on the aquatic lives in the receiving streams and vegetable farms close to the brewery plant. The temperatures of the influent wastewater was relatively high with mean values of 38°C and could have resulted from activities which included boiling of wort, pasteurization of water, washing and rinsing of returned bottles in hot and warm water respectively. High temperatures or thermal pollution can be lethal to aquatic biota as a threat to the homeostatic balance of receiving water bodies, (Jaji et al., 2007). An advantage of the oxidation pond system in this study is the reduction in the temperature values of the raw brewery wastewater in the oxidation ponds before discharge into the environment.

Foul odours in oxidation ponds are an indication of slow organic matter degradation and anaerobic decomposition at the bottom of the pond which produces mercaptans, amino acids, H_2S, NH_3 and CH_4. As the results revealed, the phosphate removal efficiencies of the oxidation ponds were poor. Several methods for the removal of phosphorus in wastewaters by oxidation pond system have been suggested. These include increasing the number of maturation ponds, so that progressively more and more phosphorus becomes immobilized in the sludge as well as chemical precipitation through the addition of chemicals such as lime, alum and iron (Tchobanoglous et al., 2003). Apart from the offensive odors from these ponds, the discharge of coloured wastewater from the ponds into aquatic environment is not only aesthetically displeasing, but also impedes light penetration, damages the quality of the receiving streams and may be toxic to food chain organisms and aquatic life (Mahdavi et al., 2001).

The concentrations of heavy metals in the raw wastewater were considerably high. For instance, the high concentration of mercury in the raw wastewater could be the result of the chemicals and equipments being used in brewing and bottling activities, while high level of iron might be from pipes and sewers that carried both raw and treated water. The efficiency of the oxidation ponds in the removal of these metals was low and the continuous discharge of high concentrations of these heavy metals can render any receiving water bodies unsafe for drinking or other agricultural purposes. Ogunfowokan et al., (2008) reported that effluent discharge from sewage ponds into receiving stream led to increase in concentrations of heavy metals such as cadmium, lead, arsenic and cobalt downstream thus impacting the receiving stream negatively and posing health risk to users of such receiving streams.

The results of this study has shown that the discharged brewery effluent greatly exceeded NESREA's (National Environmental Standards and Regulations Enforcement Agency) stipulated limits for industrial effluent discharge into surface waters with respect to total coliform density, biochemical oxygen demand (BOD_5), suspended solids and concentrations of mercury and lead despite the treatment in the oxidation ponds. Possible causes for the deterioration of the performance of oxidation ponds could be poor design of the pond; incomplete mixing; absence of or type of preliminary treatment; insufficient maintenance and increased organic influent loads (Barjenbrach and Erler 2005). It is likely that the size of the oxidation ponds designed for the company about two decades ago has become too small and the detention period of about 10 days originally designed can no longer be achieved. In order to have an adequately treated brewery wastewater through the use of the oxidation ponds, it is suggested that an adequate maintenance schedule should be developed to ensure that competent personnel visits the facilities daily to ensure that the plant is running properly. The alum-dosing pre-treatment tank should also be put in proper place for easy coagulation of solids. The need to expand the size of the oxidation ponds because of the increase in the brewing activities of the company and the possibility of using some aquatic weeds such as Water hyacinth *(Eichhornia crassipes)* in the oxidation ponds may be considered. The use of such aquatic weeds has been shown to enhance the efficiency of oxidation ponds with improvement in organic matter mineralization and removal of heavy metals at no extra cost (Kanabkaew and Puetpaiboon 2004; Abbasi and Abbasi, 2010). Finally, the chlorination of the effluent from the plant prior to discharge into the receiving stream will reduce the microbial loading into the environment.

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