International Journal of Scientific Engineering and Applied Science (IJSEAS) – Volume-2, Issue-5,May 2016
ISSN: 2395-3470
www.ijseas.com

THE PHYSICOCHEMICAL AND MICROBIAL CONTAMINATIONS OF DISCHARGED EFFLUENTS ON THE RECEIVING WATER BODY: A STUDY OF BREWERY "A" IN SOUTH-EASTERN NIGERIA.

[1]Nwokorie, C. C. and [2]Ike, C. C.
Department of Microbiology, Abia State University, Uturu.
Department of Biological Sciences, Rhema University, Nigeria. P.M.B 7021, Aba, Abia State

Abstract

The physicochemical and microbial contaminations of discharged effluents from brewery "A" located in the South-Eastern part of Nigeria and the impact on the receiving water body was assessed. The effluent flow rate, physico-chemical and microbiological parameters at discharge point (DP), upstream (US) and downstream (DS) were determined using standard methods. The effluent from brewery "A" had mean values for the following: flow rate -1032.82m^3/day, pH-11.5±0.91(DP), 6.6±0.03(US), 7.8±0.07(DS), temperature- 32±2.01(DP), 27±3.01(US), 29±2.22(DS), chemical oxygen demand(COD)- 2874.20mg/L±3.01(DP), 2548.6mg/L±3.21(US), 2766.20mg/L±2.15(DS), biochemical oxygen demand (BOD)- 2080.20mg/L±2.41(DP),1572.7mg/L±2.59(US),1800mg/L±2.14(DS),TSS- 165.6mg/L±0.56(DP),128.2mg/L±1.99(US), 139.30mg/L±2.06(DS), conductivity- 2945.80µS/cm±3.11(DP), 1448.85µS/cm±3.02(US), 2171.80µS/cm±2.89(DS), turbidity- 22.0NTU±0.22(DP), 10NTU±0.43(US), 15NTU±1.02(DS), dissolved oxygen (DO)- 11.0mg/L±0.08(DP), 23.3mg/L±2.44(US), 18.1mg/L±3.11(DS), nitrate-7.2mg/L±0.12(DP), 3.8mg/L±0.67 (US), 6.58mg/L±2.34(DS), total phosphorus- 4.60mg/L±0.04(DP), 2.18mg/L±0.76(US), 3.64mg/L±0.99(DS), and oil/grease- 8.15mg/L±0.09(DP), 2.90mg/L±1.54(US), 6.96mg/L±1.08 (DS). A total of nine microbial species were isolated from the discharged effluent and receiving water body: *Staphylococcus aureus*, *Bacillus* species, *Escherichia coli*, *Pseudomonas* species, *Klebsiella* species, *Streptococcus* species, *Saccharomyces cerevisiae*, *Penicillium* species, *and Aspergillus* species. This study indicated that discharge of effluent from brewery "A" into the water body had lead to oxygen depletion, high microbial and pollution loads and reduction of the amount of light available for aquatic vegetation.. This unsafe condition of the water body with high pathogenic loads may pose health hazards to users of the water body and also to the aquatic organisms especially the fish.

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Keyword: Effluent, Eutrophication, Water body, Microbial load.
*Correspondence author: E-mail: chrismacaug@yahoo.com

Introduction:

The discharge of untreated effluent from industries into receiving water body is a common practice in Nigeria. The environmental impact of these pollutants in the receiving water body has raised concern as users of these water bodies are exposed to varying number of diseases. Also, there is destruction of aquatic

lives in the polluted water body leading to foul odours. This has raised serious concern to environmental researchers. Untreated wastes from processing factories located in the cities are discharged into inland water bodies resulting in stench, discolourization and grease-oily nature of such water bodies. These wastes pose serious threat to man-kind as well as aquatic lives. The industrial effluents contain toxic and hazardous materials from the wastes that settle in water body as bottom sediments and constitute health hazards to the urban population that depend on the water as source of supply for domestic uses [1].

Brewing companies use up a lot of water during preparation and production of beer and malt drinks. This has equally generated the discharge of large volumes of wastewater. Microorganisms gradually break down the organic components of this wastewater produced during beer and malt drinks productions that has consumed available oxygen and subsequently pollutes water body like rivers, lakes, streams and deep-water. Brewery effluents are high in carbohydrate, nitrogen and cleaning/washing chemicals which have been proved to be serious water pollutants. The introduction of wastewater, high in organic matter and essential nutrients bring about changes in the microflora. [6] reported high count of bacterial population in Ikpoba river in Benin City, Nigeria, after receiving a brewery industrial effluent. Similar results were reported by [7] on the effect of brewery discharge into Eziama river in Aba, Nigeria.

In Nigeria, National Environmental Standards and Regulations Enforcement Agency (NESREA) is the agency that regulates the discharge of effluents and emissions from industries into the environment by setting standards and ensuring compliance. It is therefore necessary to ascertain the level of compliance to discharge standards by some of these industries.

This study was designed to determine the pollution loads (physicochemical and microbial contaminations) from Brewery "A" and impacts of the pollutants on the receiving water body.

Materials and methods:

Study area

Brewery "A" is situated in one of the states in the south-eastern geopolitical zone of Nigeria.

Collection and analysis of water samples

Wastewater samples (effluent) were collected into sterilized bottles. At each collection point, water samples were taken in separate sterilized universal bottles for microbial analysis. The bottle was grasped at the base with one hand and mouth was plunged down into the water to avoid introducing surface scum. The mouth of the bottle was positioned into the current away from the hand. The sampling depth was 15 - 30 cm below the water surface. After removal of the bottle from the water, small portion of the sampled water was poured out to allow air space of 2.5 - 5 cm for proper mixing of the sample before analysis. Then the bottles were transferred to the laboratory. Temperature of the water was measured using HANNA HI© 2030 – Potable Multiparameter Meter on the spot. The samples were transported in an icebox with sufficient ice blocks to maintain the temperature around 4 - 6ºC. The samples were then stored at 4ºC at the refrigerator in the laboratory until use. All water samples for microbiological analysis were analyzed within twenty four (24) hours after collection. The water samples for physicochemical analysis were analyzed within one (1) week of collection.

Microbiological analysis:

Preparation of wastewater sample (serial dilution)

One (1) milliliter of effluent samples was aseptically introduced into 9 ml of sterile distilled/ peptone water (10^{-1} dilution) in a test tube. Serial dilutions of the homogenates were made to 10^{-2} and 10^{-3} and each dilution was plated in replicate.

Inoculation of media

After tenfold serial dilution of each sample, using either sterile distilled/ peptone water as the diluent, 0.2ml of the sample taken from an appropriate dilution factor (10^{-2} - 10^{-3}) was inoculated onto different culture media using the spread plate technique as described by [4]. Streaking technique was adopted in the subculture to obtain pure isolates. The pure isolates obtained were inoculated into agar slants, incubated and stored as stock cultures for biochemical tests.

Determination and characterization of total heterotrophic bacterial count (THBC) from the wastewater samples.

The total heterotrophic bacterial counts of the water samples were carried out. From the serially diluted samples ranging from 10^{-3} to 10^{-6}, 0.2ml aliquots was collected and inoculated onto Nutrient agar using spread plate method as described by [4]. The inoculated plates were incubated for 48 hours at 37^0C. The colonies formed on the plates were counted and pure cultures of bacterial isolates were identified using cultural, morphological and biochemical characterization. Identification of the bacteria to genera level was based on the schemes of [3].

Determination and characterization of total coliform count (TCC) from the wastewater samples.

The method of [4] was used. The serially diluted water samples were used. 0.2ml aliquots was collected and inoculated onto Endo agar using spread plate method. The inoculated plates were incubated for 48 hours at 37^0C. The colonies formed on the plates were counted and pure cultures of bacterial isolates were identified using cultural, morphological and biochemical characterization. Identification of the bacteria to genera level was based on the schemes of [3].

Determination and characterization of total fungal counts of the wetland samples.

The method of [4] was used. The serially diluted water samples were used. 0.2ml of the diluted aliquot was inoculated using spread plate method onto Sabauroud's Dextrose Agar (SDA) fortified with 0.05mg/l chloramphenicol to suppress the growth of bacteria. The plates were incubated at temperatures between 25^0C – 30^0C for 3 – 7 days after which the fungal colonies were counted. The purified fungal isolates were identified on the basis of macroscopic and microscopic characteristics by slide culture technique, and lactophenol staining. The schemes of [2]; [10] were used for the identification.

Physicochemical studies

The physicochemical parameters measured include pH, temperature, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), conductivity, turbidity, dissolved oxygen (DO), nitrate, total phosphorus, and oil/grease. They were determined using standard methods as described in [12].

Measurement of effluent flow rate.

Readings were taken and calculated from a digital flow meter installed at the effluent discharge pipe and recorded as volume of effluent discharged in m^3 per day (m^3/day)

Results:

Table 1: The effluent parameters with reference to NESREA Standards at mean daily flow of 1032.82 m^3/day.

Parameters	Discharge Point (DP)	Upstream (US)	Downstream (DS).	Standard (NESREA)
pH	11.5 ± 0.91	6.6 ± 0.03	7.8 ± 0.07	6-9
Temperature	32 ± 2.01	27± 3.01	29 ± 2.22	40
COD (mg/l)	2874.20 ±3.01	2548.6 ± 3.21	2766.20 ± 2.15	60-90
BOD (mg/l)	2080.20 ± 2.41	1572.07 ± 2.59	1800.70 ± 2.14	30-50
TSS (mg/l)	165.6 ± 0.56	128.2 ± 1.99	139.30 ± 2.06	25
COND (µS/cm)	2945.80 ±3.11	1448.85 ± 3.02	2171.80 ± 2.89	N/S
Turbidity (NTU)	22.0 ± 0.22	10 ± 0.43	15 ± 1.02	5
DO (mg/l)	11.0 ± 0.08	23.3 ± 2.44	18.1± 3.11	N/S
Nitrate (mg/l)	7.2 ± 0.12	3.8 ± 0.67	6.58 ± 2.34	10
Total Phosphorus (mg/l)	4.60 ± 0.04	2.18 ± 0.76	3.64 ± 0.99	2.0
Oil/grease (mg/l)	8.15 ± 0.09	2.90 ± 1.54	6.96 ± 1.08	10

Values are given as mean ± SD, COD: Chemical oxygen demand, BOD: Biochemical oxygen demand, COND: Conductivity, DO: Dissolved oxygen, N/S: No Standard. [8].

Table 2: Mean microbial load.

Parameter	Discharge Point (DP)	Upstream (US)	Downstream (DS)	Standard (NESREA)
THC (cfu/ml)	7.2×10^4	6.8×10^4	6.9×10^4	N/S
Y&MC (cfu/ml)	3.3×10^3	2.9×10^3	3.1×10^3	N/S
TCC (cfu/ml)	1.8×10^3	2.0×10^3	2.2×10^3	400cfu/100ml

THC: Total heterotrophic count, Y&MC: Yeast and mold count, TCC: Total coliform count, N/S: No standard. [8].

Table 3: Prevalence of isolated microbial species.

Parameter	Discharge Point (DP)	Upstream (US)	Downstream (DS)
Staphylococcus aureus	+++	+++	+++
Bacillus species	+++	+++	+++
Escherichia coli	+	++	+++
Pseudomonas species	+++	+++	+++
Klebsiella species	++	++	++
Streptococcus species	++	++	++
Saccharomyces cerevisiae	+++	++	+++
Penicillium species	++	++	++
Aspergillus species	++	++	++

The effluent from brewery "A" had a mean daily flow rate of $1032.82 m^3$/day. The concentration of the discharged effluent parameters measured with the exception of nitrate as shown (in Table 1) were significantly higher than the NESREA permissible limits. The concentration of oil/grease was high at the discharge point (8.15 mg/l ±0.09) when compared with results from other sampling points {upstream (2.90 mg/l ±1.54) and downstream points (6.96 mg/l ±1.08) in the water body}. Although, these results were within NESREA permissible limits. pH values were higher than NESREA permissible limits at the discharge point (11.5 ±0.91) and within NESREA permissible limits at the upstream (6.6 ±0.03) and downstream (7.8 ±0.07) points in the water body. Temperature values at each sampling points were within NESREA permissible limits (DP- 32 ± 2.01, US- 27 ± 3.01, DS- 29 ± 2.22).

Total heterotrophic count (THC) is an important parameter in determining water quality. Eutrophication resulting from overload of dissolved and suspended matters and depletion of oxygen content is associated with discharge of non compliant effluent into water bodies. The mean microbial loads of both discharged effluent and receiving water body can provide valuable statistics in monitoring various types of pollutants (Table 2). Further, it must be realized that the data obtained when compared with regulatory standards were significant. The values for microbial loads were highest at the discharge point (THC- 7.2×10^4, Y&MC- 3.3×10^3, TCC- 1.8×10^3), moderate at the downstream (THC- 6.9×10^4, Y&MC- 3.1×10^3, TCC- 2.2×10^3) and with the least values at the upstream point of the water body

(THC- 6.8 x 10^4, Y&MC- 2.9 x 10^3, TCC- 2.0 x 10^3). The prevalence of isolated microbial species was shown in Table 3. A total of nine microbial species were isolated and include *Staphylococcus aureus*, *Bacillus species*, *Escherichia coli*, *Pseudomonas species*, *Klebsiella species*, *Streptococcus species*, *Saccharomyces cerevisiae, Penicillium species and Aspergillus species.*

Discussion:

The effluent from brewery "A" had posed serious environmental consequences when discharged into the receiving water body. With pollution parametric values exceeding stipulated regulatory standards thresholds (NESREA standards), the water body had been depleted of dissolved oxygen which could lead to noxious conditions as reported by [9]. The stimulation of aquatic plant growth and subsequent eutrophication of the water body resulting in depletion of dissolved oxygen and subsequent fouling from the water body was experienced. Also, breakdown of spent yeast, wort, trub and kieselguhr by microorganisms could result in depletion of dissolved oxygen concentration needed to sustain aquatic life in the water body, which could cause both foul odour and eventual death of living organisms in the water body as confirmed by [9].

High concentration of suspended solid with a mean value of 165.0mg/l ± 0.56 at the discharge point, as shown in (Table 1) was as a result of discharge of spent grain, kieselgulur, surplus yeast, trub and label pulp during production activities of brewery "A". This could contribute to the darken appearance of the water body, thereby reducing the amount of light available for aquatic vegetation, algae and mosses to photosynthesize.

The presence of phosphate in the water body could be attributed to the chemicals used and discharged during cleaning-in-place (CIP) like phosphoric acid. The discharge of phosphate had encouraged vegetation growth along the banks and eutrophication. Plankton and algal became abundant and dead plant and animals accumulated at the bottom of the water body.

Fatty organic materials from petroleum were not quickly broken down by bacteria and had caused environmental pollution. The discharged oil/grease load of 8.15 mg/l ± 0.09 from brewery "A" into the water body would form a trap/ coat, an unconducive environment for the survival of aquatic lives and other living organisms, causing foul odours. The low values obtained with dissolved oxygen (DO) indicated falls in oxygen content of the effluent and water body. Aquatic lives under this condition would be subjected to stress, which will result in mortality as reported by [5]. High microbial loads at various sampling points were above thresholds, an indication of the unsafe condition of the effluent and water body. This would discourage users as they would be exposed to diseases. The results obtained in this study are in agreement with the research reports of [9]; [1]; [7]; [6]; and [5].

Conclusion

The discharge of untreated effluent into the water body had led to oxygen depletion, eutrophication, reduction of the amount of sunlight available for aquatic vegetation and increased microbial loads with subsequent foul odour. Therefore, there is urgent need by regulatory agencies to mandate industries with the treatment of wastewater to meet stipulated standards before discharge into water bodies. This measure will ensure safe environment for both aquatic and human lives.

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