Bioremoval of Heavy Metals from a Nigerian Brewery Wastewater by Bacterial Application

Avemaria Obasi[1]* and Ogochukwu Agwu[1]

Abstract

The high demand for brewery products, as well as the technological advancement in this regard, has further accelerated the growth of these industries; and the waste generated is one of the major sources of industrial waste hazards. In this study, the effect of bacterial treatment on the heavy metal concentrations in treated and untreated wastewaters from a brewery industry located in Lagos, Nigeria was analysed. The two bacterial isolates *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were selected after painstaking isolation and purification of numerous bacterial isolates from the wastewaters using standard methods. The bacteria isolates were used individually and in combination to assay heavy metal removal in the wastewater samples during 14 days culture. The organisms grew luxuriantly, and completely removed Copper and Lead while the concentration of Zinc, Manganese and Iron reduced by between 57% and 97% for all the treatments. The study confirmed that bacteria can be applied in the removal of priority pollutants such as heavy metals from brewery wastewaters. It further demonstrates that wastewater indigenous organisms can actually perform this remediation activity if given the opportunity. This will serve as a low-cost form of wastewater treatment strategy which will tremendously reduce the concentration of the toxic heavy metals introduced into the environment and prevent bio-accumulation of these pollutants.

Keywords: heavy metal, bacteria, brewery wastewater

1. Introduction

Food processing operations produce highly concentrated organic waste; the brewery industry is one of the largest producers of industrial wastewater alongside distillery and winery industries, this being because it is one of the largest volume water users (Guthrie, 1980; Noorjahan and Jamuna, 2012). The Biochemical Oxygen Demand levels are quite high, as are the total solids, typically about half the BOD and over 90% of the suspended solids are generated in the brewing operation (Noorjahan and Jamuna, 2012). These industrial wastewaters are also the main source of heavy metals in sewage sludge, since nearly all industrial by-products consists of some level of heavy metals (Ninnekar, 1992; Olowu *et al.*, 2012 and Malu *et al.*, 2014).

[1] Department of Microbiology, University of Lagos, Lagos, Nigeria

* Corresponding authors, e-mail: avemariaobasi@yahoo.com

Brewery wastewater consists of less heavy metal concentrations. These heavy metals get into brewery through the grains which are used as raw materials, since they are usually cultivated with herbicides, fungicides and bactericides that contain heavy metals. When these grains are used as raw materials for beer production, these toxic metals pass through the brewery processes and most times, majority are distributed into the waste (Čejka *et al*., 2011). Disposal of such effluent without any prior treatment into water courses causes serious pollution problems. Liquid effluents on percolation into shallow wells through streams and lakes could be a direct source of contamination to portable water. This constitutes to the danger of public health as a result of the consumption of polluted water by the unsuspecting public, the aquatic as well as the land animals (Nikoladze *et al*., 1989; Ninnekar, 1992; Mala and SaravanaBabu, 2006; Olowu *et al*., 2012; Malu *et al*., 2014). It is however worth noting that the low concentrations of heavy metals in the wastewater and subsequently in the aquatic environment, does not necessarily suggest absence of any adverse ecological effects. It is important to know that trace elements and heavy metals such as Cd, Pb, Hg, As, Cr, Cu, Ni, Zn, B, Se and Mo can bioaccumulate and the presence of one metal can significantly affect the impact that another metal may have on an organism. They usually have synergistic or antagonistic effect (Dawodu and Ajanaku, 2008; Olowu *et al*., 2012).

Implementation of ISO 14001 certification and more stringent environmental legislation have been important factors for raising awareness in the brewing industry towards effluent fluid control (Iyang, Bassey and Iyang, 2012). The pressure from these statutory and environmental law enforcements to minimize pollution necessitates that effective treatment of brewery effluents is carried out prior to discharge into receiving water body. Hence there is need to employ variety of treatments for various components of the wastewater including the heavy metals (Dawodu and Ajanaku, 2008). Treatment of brewery effluent is a very important consideration before its disposal and biological treatment process involving the use of microorganisms has been known to remove biodegradable organics, suspended solids and pathogens. Treatment of industrial effluents using microorganisms like *Eichhorniasp and Pernaviridis* have been carried out by many researchers (Sridevi, 2000; Bhavani, 2000; Adekunle and Oluyode, 2005; Aftab and Noorjahan, 2006, Noorjahan and Jamuna, 2012). Microorganisms have also demonstrated the ability to extract metals through biolixiviation, bioaccumulation and bioadsorption. These are regarded as economically viable alternatives to existing methods and can also be used in combination with other techniques (Omar *et al*., 1997). Therefore, in this study, an attempt was made to evaluate the efficiency of bacterial isolates from a brewery effluent to remove the heavy metal components of the wastewater.

2. Materials and Methods

2.1 Wastewater Sampling

Wastewater samples from a brewery industry located in Lagos, (GPS coordinates: N06° 42^{1} 21.4”; E003° 13^{1} 44.8”) Nigeria were collected in sterile plastic containers. The untreated and treated wastewaters were collected upstream before treatment and downstream after treatment respectively from the treatment plant. The samples were stored in ice packs and immediately transferred to the laboratory for heavy metal and routine microbiological analyses.

2.2 Isolation and Identification of Bacteria from Brewery Wastewaters

As a preliminary study, bacteria were isolated from the wastewaters using Nutrient agar medium according to standard microbiological methods described by Nwachukwu and Akpata (2003). These bacterial isolates were further screened based on their relative abundance and environmental friendliness. Two outstanding isolates were then selected for removal of heavy metal from the brewery wastewaters. The isolates were further identified on the basis of their colonial morphology, cellular morphology and biochemical characteristics according to the method of Barrow and Feltham (1995) and also by the reference to Bergey’s Manual of Determinative Bacteriology (Holt *et al*., 1994).

2.3 Determination of the Physicochemical Parameters and Heavy Metal Content of the Brewery Wastewater

The pH of the water samples were determined by the electronic method using a mettle Toledo Seven Easy pH meter. The total alkalinity, total acidity and total hardness were measured by the titrimetric method (Association of Official Analytical chemist, 1990). Phosphate, Nitrate and Sulphate were determined by standard methods using the spectrophotometer (model spectronic 20^{D+}). Winklers method (by Alsterberg) was used for Dissolved Oxygen and the Biochemical Oxygen demand and total dissolved solids estimated using whatman GF/C grade (glass filter paper). The metal ions in the water samples were determined using the appropriate lamps of flame atomic absorption spectrophotometer (AAS; model: Analyst 200 Perkins Elmer) as described byAgilent Technologies Inc. (2012).

2.4 Treatment of Brewery Wastewater with Bacterial Isolates

Freshly prepared cultures of the two selected bacteria (Kbe and Pbe) were suspended in sterile phosphate buffer solution (0.005g Na_2HPO_4 and 0.301g KH_2PO_4 per 1000ml of distilled water) to attain an optical density (OD) of 0.5 at a wavelength of 620nm. This was equivalent to a total viable count of $7.8x10^{8}$cfuml^{-1} and $3.9x10^{9}$cfuml^{-1} for KBe and PBe respectively.

Replicate flasks each containing 200 ml of brewery wastewater were inoculated with approximately 2% (4 ml) of the bacteria KBe and PBe suspension singly and a combination of 1% KBe and 1% PBe (KPBe). Meanwhile, the control experiment was wastewater devoid of any bacterial inoculums.The inoculated wastewater samples were incubated at room temperature 27±(2)^{o}C for 14 days. The Total viable count (TVC) of bacteria was estimated by plating out appropriate dilutions of the cultures unto nutrient agar plates every two days.

3. Results and Discussion

3.1 Microbiological Analysis

The total bacterial counts on the brewery wastewaters ranged from 3.9x10^{6} to 2.12x10^{7}cfuml^{-1}. Physiological and biochemical characterization were tentatively used to identify the bacterial isolates. Bacteria isolated include *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Escherichia coli, Enterobacter Sp. and Bacillus Sp.* Two bacterial isolates, *Klebsiella pneumoniae* (KBe) and *Pseudomonas aeruginosa* (PBe), were selected based on their relative abundance and non-pathogenic trait which was negative when tested on blood agar.

3.2 Time Course Growth of Isolates on Brewery Wastewaters from Lagos, Nigeria

The microorganisms multiplied in the wastewater, this was evident as the total viable count increased, especially between day 0 to 8 for both the untreated (Figure 1A) and the treated (Figure 1B) brewery wastewaters. Generally during the fourteen days of treatment, the total viable count increased by a three order of magnitude for all treatments in the untreated wastewater samples including the control experiment. However, the increase in total viable count varied in the different treatments. The untreated wastewater had an increase from 1.8×10^{7} to 1.9×10^{9}; 1.4×10^{7} to 1.0×10^{9}; 8.9×10^{7} to 2.4×10^{9} and 2.2×10^{7} to 3.8×10^{9} for the control, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and the combination of the two bacteria respectively. Nevertheless, the total viable count obtained in the treated wastewater initially (0 to 8 days) increased but subsequently declined. During the first eight days of incubation, the control experiment increased from 7.0×10^{6} to 1.5×10^{8}, while the others *K. Pneumoniae, P. aeruginosa,* and the combination of the two bacteria showed increase from 5.6×10^{7}, 6.0×10^{7} and 1.2×10^{7} to 5.0×10^{8}, 9.3×10^{8} and 6.8×10^{7} respectively. Biological wastewater systems have been developed to use the natural ability of bacterial communities to break down and remove polluting compounds from wastewater. They are designed for the removal of oxidizable organic carbon (known as biochemical oxygen demand), oxidizable chemicals (known as chemical oxygen demand), and nutrients especially nitrogen and phosphorous, which can cause eutrophication in waterways downstream of the treatment plant

(Eland, 2016). The rate of proliferation observed among the bacteria isolates in this study particularly in the untreated wastewater during the 14 days culture shows that these isolates utilizes organic carbon and other nutrients for their growth. This will subsequently result in the reduction or even total removal of these components of the waste. It was also obvious that there were more growth nutrients in the untreated wastewater than in the treated wastewater because the former sustained the microbial growth for a longer time (Table 1).

Table 1 Mean values of physicochemical parameters and heavy metal content of the brewery wastewaters.

Parameters	Untreated	Treated	FEPA Limits
pH	4.30±0.141	8.20±0.071	6–9
Temp (°C)	27.10±0.141	26.80±0.41	≤3°C
Nitrate(mgl^{-1})	0.54±0.014	0.38±0.042	20
Phosphate(mgl^{-1})	0.10±0.000	0.02±0.000	5
Sulphate(mgl^{-1})	1.68±0.014	2.68±0.028	500
TDS(mg/l)	134±1.414	809±2.121	2000
BOD_5(mg/l^{-1})	98±0.141	50.40±0.283	30
Zinc(mgkg^{-1})	0.47±0.007	0.15±0.007	Less than 1
Lead (mgkg^{-1})	0.18±0.007	0.32±0.021	Less than 1
Nickel (mgkg^{-1})	ND	ND	Less than 1
Iron (mgkg^{-1})	1.10±0.028	1.11±0.000	20
Manganese (mgkg^{-1})	0.43±0.014	0.12±0.000	5
Copper(mgkg^{-1})	0.01±0.000	0.02±0.000	Less than 1

Note: ND mean not detected; Data represents Mean ± SD of replicate samples

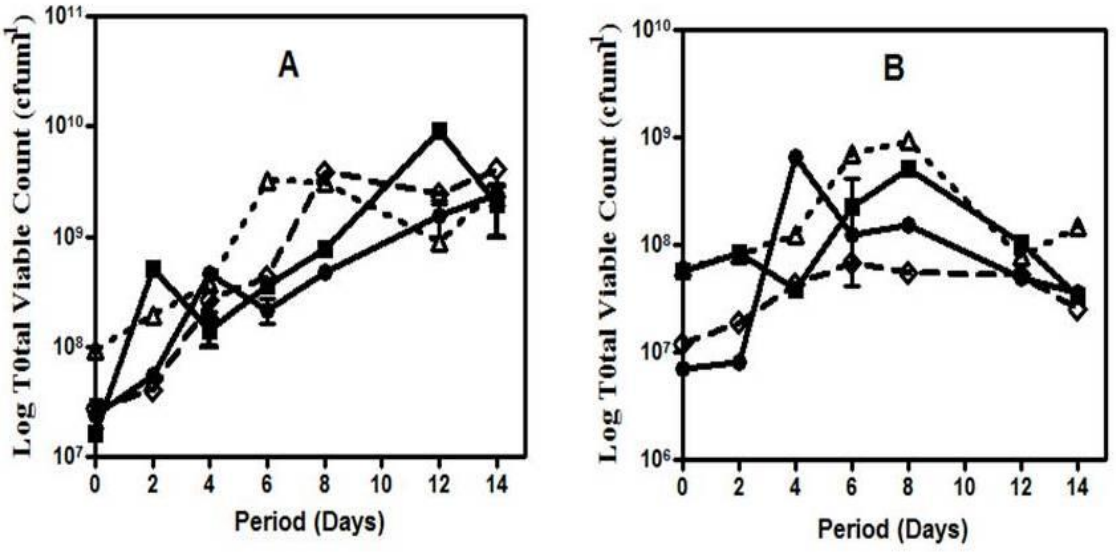

Figure 1 Total viable count of bacteria in untreated (A) and treated (B) brewery wastewater during fourteen day treatment with *K. pneumoniae* ■ ; *P. aeruginosa* △; combination of the two bacteria ◇ and control experiment ●

3.3 Heavy Metal Removal from the Brewery Wastewaters

There was an overall reduction in the concentration of heavy metals monitored in the brewery wastewater after the inoculation of the bacteria (Table 2 and 3). In the untreated wastewater, the removal of Lead and Copper was outstanding, with all the treatments resulting in complete removal of the metals. The concentration of Zinc decreased from 0.47 to 0.03mgkg^{-1} in the treatment with *K. pneumoniae,* while a reduction to concentration to 0.02 was noted for the other two treatments. About 88% of the manganese was removed by the individual bacteria while their combination removed approximately 79% of this metal. It was also noted that while the treatments *with K. pneumoniae* and *P. aeruginosa* reduced the Iron content, from 1.08 to 0.66 and 0.67mgkg^{-1} respectively, no form of reduction was observed in the treatment with the combination of the two organisms.

Similarly, in the treated brewery wastewater, Copper was not detected after fourteen days of the bacterial treatment, meanwhile Lead was also not detected in the treatment with the individual bacteria but the combination of the two bacteria removed about 71%. On the other hand, Iron was completely removed by combining the two bacteria; *P. aeruginosa* reduced the concentration by 97% while *K. pneumoniae* removed 57%. The concentration of Zinc and Manganese were also tremendously reduced by all the treatments although in varying proportions. In this present study, the ability of bacterial isolates to remove six heavy metals namely; Zinc, Lead, Iron, Manganese, Copper and Nickel from brewery wastewaters was attempted. Contrary to an earlier study on effluents from industries in Ikeja Lagos, which reported that, the concentration of heavy metals in effluent discharge is on the high side exceeding the maximum recommended limits (Sangadoyin, 1995). Analysis of the wastewater in this study, revealed that the concentration of these metals were below the recommended standards by FEPA (1991), {Now LASEPA (Lagos State Environmental Protection Agency)} and World Bank (1997), "Industrial Pollution Prevention and Abatement for Breweries". Meanwhile, Malu et al., (2014) reported that the concentrations of Cr, Cu, Cd, Fe, Mn, Ni and Pb in brewer's spent grains obtained from a brewery in Markurdi, North central of Nigeria, were within the allowable limit for both international and national standards, except Zinc which was very high. Furthermore, Alao et al. (2010) reported 0.7mgl^{-1} of lead, while cadmium, arsenic and mercury were not detected in brewery effluent from Ibadan, Nigeria. Nevertheless, there is the concern that these pollutants unlike some other organic pollutants cannot be degraded, they persist in the environment and bio-accumulate over time. This can result to loss of aquatic life and uptake of polluted water by plants and animals, which eventually gets into human body resulting in health related problems (Dan'azumi and Bichi, 2010). After a 14 days treatment period, the bacterial isolates (*Klebsiella pneumoniae and Pseudomonas aeruginosa*) in this study demonstrated excellent heavy metal removal potentials. In the untreated wastewater, Lead and Copper were completely mopped up while the concentrations of Zinc and Manganese was tremendously reduced (Table 2). Similarly, for the treated wastewater, Lead and copper also disappeared while a reasonable reduction was noted for the other metals including Iron (Table 3). Two microorganisms *Myxococcus Xanthus* and *Saccharomyces cerevisiae* have exhibited efficient biosorption of heavy metals from a metal contaminated solution (Omar *et al*., 1997). This reduction in concentration will surely affect availability of these heavy metals in the wastewaters when eventually discharged into the environment hence preventing bioaccumulation in the biotic and abiotic ecosystems.

The concentration of Iron was highest among the metals in both the treated and untreated wastewater. Earlier study on the water quality of Ogun River in Nigeria, which receives industrial effluents from Lagos and Abeokuta had revealed high levels of Iron (Jaji, Bambgose, Odukoya and Arowolo, 2007). In this study, it was observed that the removal of the metal Iron was more effective in the treated wastewater, unlike what was noted in the untreated waste. This suggests that bacterial removal of heavy metals will be more efficient when used alongside other treatment methods. According to Jaiyeola and Bwapwa (2016), the different brewery effluent treatment methods displayed some levels of efficiency but each has its weaknesses. Hence, there is still need for an efficient low cost remediation strategy which might encompass a variety of methods.

During an evaluation of the effect of brewery effluent disposal on public water bodies in Nigeria, Dawodu and Ajanaku (2008) discovered that physicochemical conditions of surface waters at some areas in Nigeria have been influenced by the various pollutants. The bio-accumulation of heavy metals in different terrestrial biota was investigated in Kanting national park, Taiwan and high concentration of Cd, Hg and Sn was found in snail, earthworm, crab, lizard, snake and bat. Similarly, high level of Hg was found in invertebrates, amphibians and reptiles which revealed a strong influence from industrial effluent (Hsua, Selvarajb and Agoramoorthy, 2006). Consequently, the ability of any waste treatment method to completely remove these perilous pollutants from wastewater prior to discharge into the environment will be of great relevance.

Table 2 Mean values of heavy metal concentration of the untreated brewery wastewaters after fourteen days inoculation with bacterial isolates

Heavy metal concentration (mgkg^{-1})	**Control**	***K. pneumoniae***	***P. aeruginosa***	***K. pneumoniae* and *P. aeruginosa***
Zinc	0.47±0.007	0.03±0.000	0.02±0.000	0.02±0.000
Lead	0.18±0.004	ND	ND	ND
Nickel	ND	ND	ND	ND
Iron	1.08±0.000	0.66±0.007	0.67±0.007	1.74±0.005
Manganese	0.42±0.007	0.05±0.000	0.05±0.000	0.09±0.000
Copper	0.01±0.000	ND	ND	ND

Note: ND mean not detected; Data represents Mean ± SD of replicate samples

Table 3 Mean values of heavy metal concentration of the treated brewery wastewaters after fourteen days inoculation with bacterial isolates

Heavy metal concentration (mgkg^{-1})	**Control**	***K. pneumoniae***	***P. aeruginosa***	***K.pneumoniae* and *P. aeruginosa***
Zinc	0.14±0.007	0.03±0.000	0.001±0.000	0.004±0.000
Lead	0.31±0.007	ND	ND	0.09±0.000
Nickel	ND	ND	ND	ND
Iron	1.11±0.077	0.48±0.007	0.03±0.000	ND
Manganese	0.12±0.001	0.02±0.000	0.02±0.000	0.03±0.000
Copper	0.02±0.000	ND	ND	ND

Note: ND mean not detected; Data represents Mean ± SD of replicate samples

4. Conclusion

This study demonstrated that although the concentrations of heavy metals in the brewery effluents were below the recommended concentrations. The potentials exhibited by these bacterial isolates suggests that they will be very useful in the removal of heavy metals in waste with even higher concentrations and so can be incorporated into the waste treatment plan. In fact, since these organisms were isolated from the wastewater, it further implies that resident or indigenous bacteria of the wastewaters have potentials of removing some of the hazardous components of the waste if given the opportunity. This may serve as a low cost bioremediation of brewery wastewater.

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