Journal of Advances in Microbiology

22(6): 22-29, 2022; Article no.JAMB.84866
ISSN: 2456-7116

Assessment of the Effect of Electromagnetic Field on Bacterial Load and Physicochemical Properties of A Selected Food and Dairy Industry Effluent in Akure

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2022/v22i630465

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/84866

Original Research Article

Received 15 January 2022
Accepted 28 March 2022
Published 25 April 2022

ABSTRACT

Management of industrial effluents is one of the major challenges affecting developing nations of the world. Many of these industries release their effluents indiscriminately into the environment without proper treatment, which has caused a great health hazards to the environment and living population of this nation. The aim of this study was to determine the effect of electromagnetic field on microbial load of industrial effluents from food and dairy industry at Akure, Ondo state, Nigeria. Bacteria were isolated and identified using biochemical and molecular methods. Microbiological analysis and physicochemical characterization of the industrial effluent were carried out to determine the effect of electromagnetic field on microbial load and physicochemical parameters of the industrial effluents. Eleven bacteria were isolated from the effluents, the morphological, microscopic and biochemical characteristics of the eleven isolated bacteria suggests the isolates to be *Bacillus cereus, Bacillus subtilis ,Enterobacter aerogenes, Enterococcus faecalis, Lactococcous lactis, Micoccocus luteus, Pseudomonas aeruginosa, Proteus vulgaris, Rhodococcus equi, Serratia marcesans* and *Staphylococcous aureus*. It was observed that electromagnetic field (EMF) had significant effect on the bacterial load of the industrial effluent. Mean differences was considered significant at P≤0.05. Bacterial load decreases as the magnitude of the EMF increases with time. The physicochemical

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analysis of the raw and EMF treated effluents revealed that EMF treated effluent had lower mean values of physicochemical. This outcome of study shows that Electromagnetic field treatment of effluent is effective in reducing the bacterial load of food and dairy industrial effluents, hence making the effluent safe for the environment to which it will be discharged. The method for the treatment can be developed and modified for effective use in industrial settings which will be of great economic importance to food and dairy industries and waste water management of developing nations.

Keywords: Industrial effluents; electromagnetic field; food and dairy industry; microbiological analysis; physicochemical parameters; waste water management.

1. INTRODUCTION

Industrial effluents are industrial wastewaters entering a water body represent a heavy source of environmental pollution in Nigerian rivers**.** One of the major problems of developing countries of the world is poor management of vast amount of wastes generated by various anthropogenic activities [1]. The contamination of natural water bodies by Industrial effluent has been a major challenge in densely populated and developing countries such as Nigeria [2]. Wastes are been disposed in Nigeria majorly through the River systems most especially effluents from industries that are sited close to the river [3]. This effluent from industries contributes largely to the pollution of the water system. The effluent can alter the physical, chemical and biological properties of the receiving water system. Increased discharged of this untreated effluent into water bodies have led to gross pollution and contamination of surface waters both from industrial, agricultural and domestic sources [4]. Effluent management is achieved by proper collection and treatment of the effluents which has two major objectives. In which are to reduce the health risk of the environment by protecting it against air and water pollution. The second is the utilization of this water resource to mitigate scarcity of water and preserve the fresh water [5]. Electromagnetic Field has great potentials in inhibiting these microorganisms without adverse effect on the water bodies. Findings of the previous studies have demonstrated the high efficacy of EMFs as adjunctive or alternative of conventional effluent treatment. Different modalities of EMFs can improve different steps of effluent treatment process [6]. For example, EMFs can improve the activating sludge and disinfection processes. In addition, antimicrobial and antibacterial properties of different frequency bands of EMF spectrum like different intensities are useful in the effluent treatment [7]. These antimicrobial effects can be used in the disinfection process of the effluent treatment process or as a stand-alone process of effluent treatment [8]. The fundamental research fields of electromagnetic field applications in effluents treatment are magnetite slurry, magnetic particles, modifying initiated slop, and magnetic powder. The aim of this study was to determine the effect of electromagnetic field on microbial load of industrial effluents from food and dairy industry in Akure, Ondo State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

Captain cook food industry, Akure, Ondo State, Nigeria was the study location for this research, it is situated at 7.25^{o} North latitude, 5.19^{o} East longitude and 396 meters elevated above the sea level. Akure is a big town in Nigeria situated in southwestern Nigeria and is the largest city and capital of Ondo State. It has more than 556,300 inhabitants.

2.2 Collection of Effluent Samples

Effluent samples were collected from septic tanks of food industry using sterile container and transported to the laboratory for experiment. The food industry manufactures; bread, table water and has an Eatery which is located along Alagbaka. They also manufacture Pet bottles and Polythene bags of all sizes.

2.3 Characterization and Identification of Bacterial Isolate

The isolates were characterized using colonial and cellular morphology as well as biochemical reactions. They were subsequently identified using cowan and steels manual for identification of medical bacteria [9].

2.4 Molecular Identification of Bacterial Isolates Present in the Effluents After Treatment Extraction of DNA using CTAB Method

Bacteria isolates grown overnight was transferred to eppendorf tube and it was spun

down at 14,000rpm for 2 minutes, the supernatant was discarded and 600 µl of 2X CTAB buffer was added to the pellet and it was incubated at 65 ˚C for 20mins. The sample was removed from the incubator and allowed to cool to room temperature and chloroform was added the sample was mixed by gently inversion of the tube several times. Thereafter, the sample was spun at 14,000 rpm for 15 minutes and the supernatant was transferred into a new eppendorf tube and equal volume of cold Isopropanol was added to precipitate the DNA. The sample was kept in the freezer for 1hour and later spun at 14,000rpm for 10 minutes and the supernatant was discarded and the pellet was washed with 70% ethanol later the sample was air dried for 30mins on the bench. The pellet was re-suspended in 100ul of sterile distilled water. DNA concentration of the samples was measured on spectrophotometer at 260 nm and 280 nm and the genomic purity were determined. The genomic purity was between 1.8 –2.0 for all the DNA samples [10].

2.4.1 DNA electrophoresis

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0% agarose gels. Agarose gels were prepared by dissolving and boiling 1.0g agarose in 100 ml 0.5 X TBE buffer solutions. The gels were allowed to cool down to about 45˚C and 10 µl of 5 mg/ml ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel had solidified, 3 µl of the DNA with 5 µl sterile distilled water and 2 µl of 6X loading dye was mixed together and loaded in the well created. Electrophoresis was done at 80V for 2 hours. The integrity of the DNA was visualized and photographed on UV light source [10].

2.4.2 PCR analysis using I6S primer

PCR analysis was run with a universal primer for bacteria called 16S. The PCR mix comprises of 1 µl of 10X buffer, 0.4 µl of 50 mM MgCl2, 0.5 µl of 2.5 mMdNTPs, 0.5µl 5 mM Forward primer, 0.5µl of 5mM Reverse primer, 0.05µl of 5units/µlTaq with 2µl of template DNA and 5.05µl of distilled water to make-up 10µl reaction mix. The PCR profile used is initial denaturation temperature of 94˚C for 3mins, followed by 30 cycles of 94˚C for 60sec, 56˚C for 60sec, 72˚C for 120 seconds and the final extension temperature of 72˚C for 5 minutes and the 100˚C hold forever [10].

2.4.3 Purification of PCR products

The amplicon is further purified before the sequencing using 2M Sodium Acetate wash techniques. To about 10µl of the PCR product, add 1µl 2M NaAct pH 5.2, followed by 20µl Absolute Ethanol, keep at -200˚C for 1hr, spin at 10,000rpm for 10 mins, then wash with 70% ethanol and air dried. Resuspended in 5µl sterile distilled water and keep at 40˚C for sequencing [11].

2.4.4 PCR for sequencing

The primer used for the reaction was forward I6S. The PCR mix used includes 0.5µl of BigDye Terminator Mix,1µl of 5X sequencing buffer, 1µl of M13 forward primer with 6.5µl Distilled water and 1µl of the PCR product making a total of 10µl. The PCR profile for Sequencing is a Rapid profile, the initial Rapid thermal ramp to 96˚C for 1min followed by 25 cycles of Rapid thermal ramp to 96˚C for 10 seconds Rapid thermal ramp to 50˚C for 5 seconds and Rapid thermal ramp to 60˚C for 4 minutes, then followed by Rapid thermal ramp to 40˚C and hold awhile [11].

2.4.5 Purification of PCR sequencing products

The PCR sequence product is also purified before the sequencing running using 2M Sodium Acetate wash techniques. To 10µl of the PCR product add 1µl 2M NaAct pH 5.2, then add 20µl Absolute Ethanol, keep at -200C for 1hr, spin at 10,000rpm for 10 mins, then wash with 70% Ethanol and airdried. Re-suspend in 5µl sterile distilled water and keep at 40˚C for sequencing running [11].

2.5 Microbiological Analysis of Raw and Treated Effluents

Microbiological analysis was carried out on all samples. The triplicate values of the bacteria colony count for each sample was counted and recorded.

2.6 Physicochemical Analysis of Effluent Sample

Physicochemical Characterization was carried out at 0 hours i.e. before exposure and 144 hours after exposure. Physicochemical parameters of the effluent samples were assessed using [12] method. The

physicochemical parameters were temperature (oC), pH, conductivity (ΩSCM), turbidity (NTU), dissolved oxygen (mg/L), total solids (mg/l), total dissolved solids (mg/L), chloride ion (mg/L), phosphate (mg/L), Sulphate (mg/l), Nitrate (mg/L), Potassium (mg/L), hardness (mg/L), biochemical oxygen demand (BOD mg/L), Chemical oxygen demand (COD mg/L) and nitrate (mg/L). Standard guidelines of effluent sampling and physicochemical parameters evaluation was adopted [13].

2.7 Statistical Analysis

Experimental numerical data were subjected to Analysis of Variance (ANOVA) and mean separated using Duncan's New Multiple Range test using Minitab 17.0 version. Mean differences was considered significant at P≤0.05.

3. RESULTS

3.1 Microscopy and Biochemical Tests for the Bacteria Isolated in the Effluents from Food Industry

The morphological, microscopic and biochemical characteristics of the eleven isolated bacteria suggests the isolates to be *Bacillus cereus, Bacillus subtilis ,Enterobacter aerogenes, Enterococcus faecalis, Lactococcous lactis, Micoccocus luteus, Pseudomonas aeruginosa, Proteus vulgaris, Rhodococcus equi, Serratia marcesans* and *Staphylococcous aureus*

3.2 Distribution of Bacteria in the Food and Dairy Industry Effluents

A total number one hundred and forty one bacteria were isolated and biochemically identified *Bacillus cereus, Bacillus subtilis ,Enterobacter aerogenes, Enterococcus faecalis, Lactococcous lactis, Micoccocus luteus, Pseudomonas aeruginosa, Proteus vulgaris, Rhodococcus equi, Serratia marcesans* and *Staphylococcous aureus*.with varying frequency from the food and dairy industry's effluents with *Bacillus cereus* having the highest percentage occurrence at 30.9% and *Rhodococcus equi* having the lowest percentage occurrence at 1.75% (Table 1).

3.3 Effect of EMF at Different Intensities and Time Intervals on Bacterial Load of the Food and Dairy Industry's Effluents

It was observed that the treatment of the food and dairy industry effluents with electromagnetic field (EMF) had significant effect on the bacterial load of the effluent. Mean differences was considered significant at P≤0.05. Bacterial load decreases as the magnitude of the EMF increases with time. At magnitude of 130 nano Tesla (nT) of the EMF the bacterial load was reduced from 155 cfu/ml to almost zero cfu/ml at 144hrs while at magnitude of 70 nano Tesla (nT) of the EMF the bacterial load was reduced from 155 cfu/ml to 37 cfu/ml (Fig. 1).

3.4 Physicochemical Analysis of Raw and EMF Treated Effluent from the Food and Dairy Industry

The physicochemical analysis of the raw and EMF treated effluents revealed that EMF treated effluent had lower value of Physicochemical (Cond = Conductivity, T = Total, DO= Dissolve oxygen BOD=Biological oxygen demand, COD=chemical oxygen) compare to raw effluents while there temperature remain the same for raw and EMF treated effluents. This shows that EMF treatment lowers the physicochemical properties of the effluent (Fig. 2).

Table 1. Distribution of bacteria in the food and dairy industry

Probable isolates	Total number of organisms	Percentage of occurence
E. aerogenes	13	9.21
P.aeruginosa	8	5.67
M. luteus	12	8.48
B. cereus	47	33.31
R. equi	2	1.75
S. aureus	26	18.43
S. Marcesans	9	6.28
E. faecalis	19	13.27
P. vulgaris	5	3.6
Total	**141**	**100**

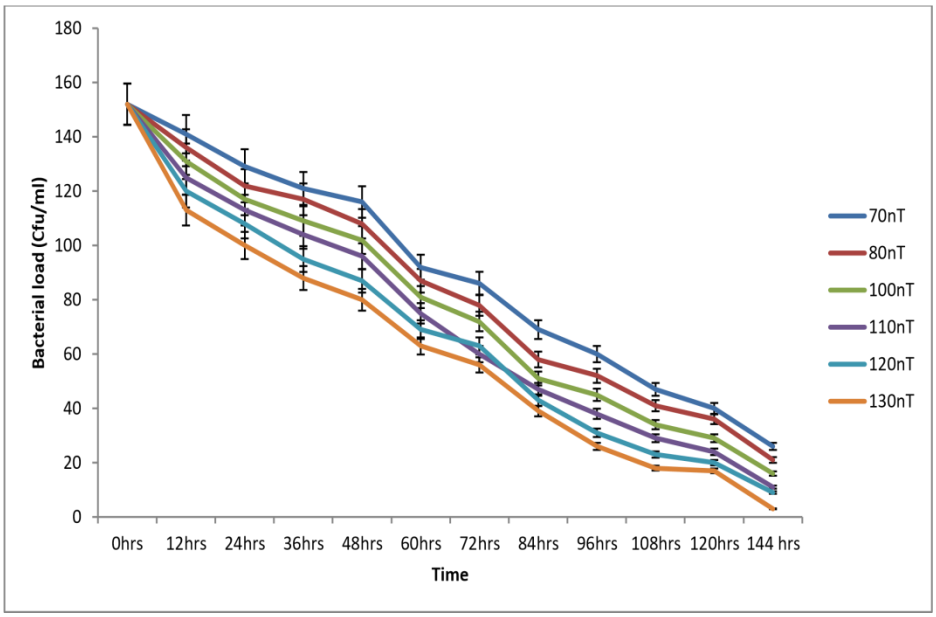

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Fig. 1. Effect of EMF at different intensities and time intervals on bacterial load of the Food and Dairy Industry's Effluents

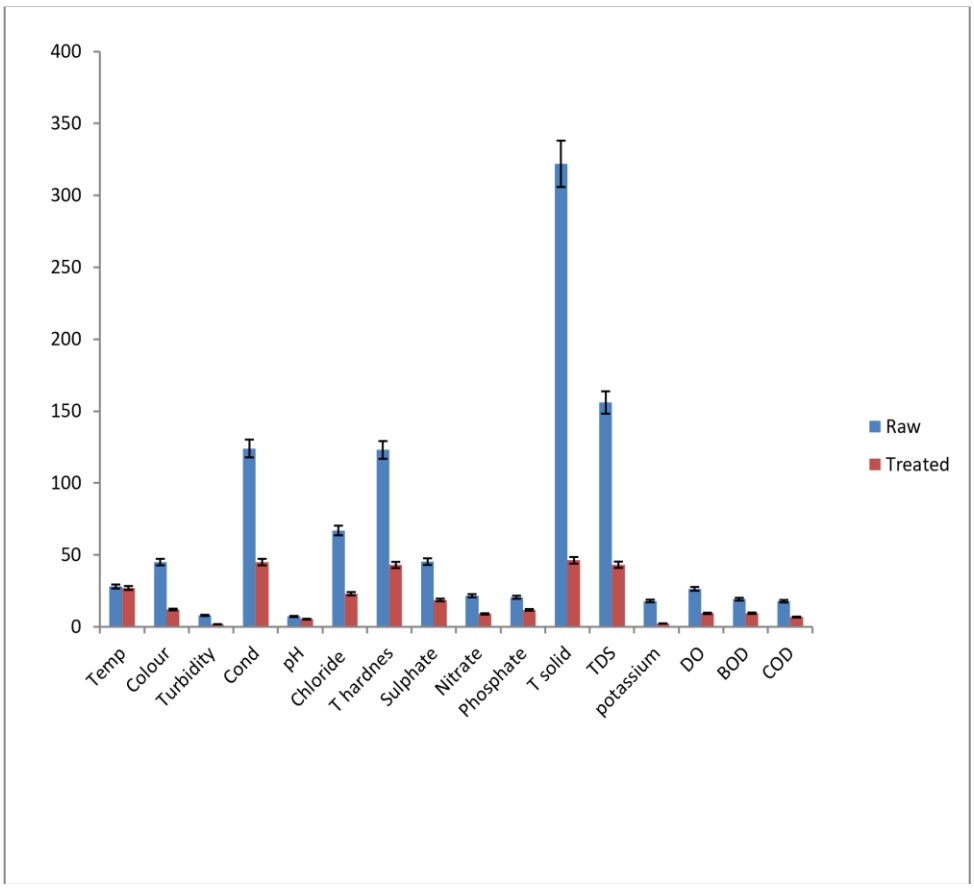

Fig. 2. Physicochemical Analysis of Raw and EMF Treated Effluent from the Food and Dairy Industry

Table 2. Molecular identification of bacterial isolates present in the effluents after treatment

Isolate identity	Accession	% identity
Staphylococcus aureus	AP017922.1	82.55%
Bacillus cereus	CP020437.2	100%
Enterococcus faecalis	CP028285.1	98.59%

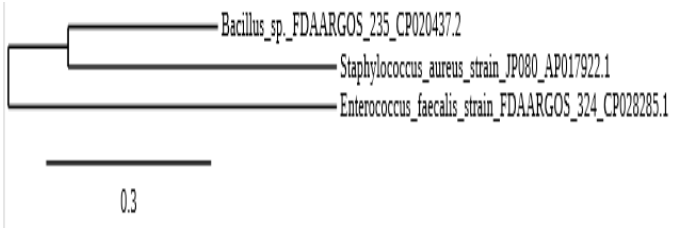

Fig. 3. Phylogenetic tree the molecularly identified bacterial isolates present in the effluents after treatment

*Legend: Temp= Temperature, Cond= Conductivity, T = Total, DO= Dissolve oxygen BOD=Biological oxygen demand, COD=chemical oxygen demand,*Significant p < 0.05*

3.5 Molecular Identification of Bacterial Isolates Present in the Effluents after Treatment

The bacteria isolates were molecularly identified as *Staphylococcus aureus* - AP017922.1, *Bacillus cereus* - CP020437.2, and *Enterococcus faecalis* - CP028285.1 with similarity of 82.55% 100% 98.59% respectively with what has been deposited in gene data base (Table 2). The Phylogenetic tree revealed that the identified bacterial isolates were similar genetically having 0.3 of generational interval (Fig. 3).

4. DISCUSSION

The presumptive identified bacteria (*L*. *lactis, E*. *aerogenes, P. aeruginosa, M*. *luteus*, *B*. *cereus, R*. *equi, S. aureus, S*. *Marcesans, E*. *faecalis, B*. *subtilis, P*. *vulgaris*) isolated from the industrial effluents suggested that the effluent is grossly contaminated with faecal and pathogenic bacteria [14]. This might be as a result of accumulated effluents from industrial cleaning, run off water from staff's rest rooms, water from products inputs washing/cleaning and accumulation of runoff water from food and dairy processing department which might contain pathogenic bacteria or contain nutrients that support the growth of the bacteria. This is related to the findings of [15] in which they detected gross bacterial contamination of milk and dairy industry processing water due to unhygienic practices during collection and storage of the water. The effluents will be hazardous to public health if is been released to the environment without been treated [16]. This can actually cause a fish kill if the effluents is released into natural water bodies without pretreatment [17]. It can also stir up an epidemic [4]. *B. cereus* having the highest percentage occurrence at 33.31% may be due to the fact that dairy products has high nutrient content which can serve as good substrate for contaminating bacteria such as *B. cereus* and more importantly *B. cereus* can survive industrial pasteurisation. This claim is related to the findings of [18] in which they attributed high occurrence of *B. cereus* in dairy industry to the ability of *B. cereus* to survive pasteurisation and high nutrient content of dairy products which serves as good substrate to contaminating bacteria such as *B. cereus*.

The effect of EMF treatment on the bacterial load of the industrial effluents shows that the application of EMF treatment reduces the bacterial load of the food and dairy industry effluents to a bare minimum. This is related to the findings of [19] in which they discussed the potentials of electromagnetic field for treatment of water and industrial wastewater which inhabits abundance of pathogenic bacteria. This also align with the assertion of [20], in his publication, which he titled "The electrical conductivity of bacteria, and the rate of sterilisation of bacteria by electric currents" says and we coat "Electrical currents, both alternating and direct, retard the growth of bacteria in liquids through which they are passed, and under certain conditions cause complete sterilisation. The cell-contents are coagulated by the heat generated, or by electrolytic effects within or without the cell.

There is the further possibility that protoplasm may be disintegrated by the mechanical action of an alternating current upon molecular charges, similar in effect to that of rapid vibration, which is known to check the growth of, and even to kill, bacteria. Whether the retardation of growth is regarded as the result of changes in the cell or liquid, the effect is largely controlled by the relation between their electrical conductivities".

In the physicochemical parameters, there was decrease in temperature of treated sample which was 25^0 C while the raw sample was 27^0C. The colour of raw sample was 46 when treated it reduced to 24. It shows that the treatment is effective in removing the excess colour from the waste water. This is related to the findings of [21] which they reported colour removal from textile waste water after been treated by adsorption process using activated carbon derived from rice husk. The turbidity of raw sample was 13.54 NTU; it decreased to 6.98 NTU when treated. The electrical conductivity of raw sample was 258 ΩSCM and decreased to 79 ΩSCM when treated. The P^H of raw sample was 6.52 and 5.78 when treated. The chloride of raw sample was 63 mg/L while the treated was 28 mg/L. The biochemical oxygen demand was determined, the value of raw sample was 18.67 mg/L and the value of treated sample was 13.58 mg/L. The lower values recorded for the physicochemical parameters of EMF treated effluents compare to raw effluents i.e. untreated effluents which had higher values of physicochemical parameters can be attributed to effect of EMF on the effluent components. This is in accordance with the findings of [22] in which magnetic treated irrigation water had lower value of physicochemical parameters compare to untreated irrigation water. The molecular identification of isolates after treatment showed that the treatment was effective in reducing the number and load of bacteria present in the effluents.

5. CONCLUSION

The effluents from the food and dairy industry was grossly contaminated with pathogenic bacteria in which *B. cereus* was observed to have the highest percentage occurrence hence there is a need for treatment of this effluence before releasing into the water bodies or the environment. Electromagnetic field treatment proved to be effective in reducing the load of isolated pathogenic bacteria from food and dairy industrial effluents. EMF also had significant effect on physicochemical parameters of effluents by lowering its physicochemical values compare to untreated effluents. This suggests EMF can be used in treatment of chemically contaminated effluents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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