



Impact of Electromagnetic Field on Bacterial Population and Physicochemical Properties of Cassava Wastewater

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ABSTRACT: The release of industrial wastewaters into the environment is a major source of toxic contaminants causing global health and environmental challenges. This study aimed to investigate the impact of electromagnetic field (EMF) treatment on bacterial population and physicochemical characteristics of cassava wastewater. Cassava wastewater sample was collected from point source of a small-scale cassava industry in Akure, Nigeria. Sample was thereafter treated with EMF of varying intensities (70- 130nT) and bacterial population and physicochemical properties of raw and treated wastewater sample were determined using standard microbiological, physical and chemical analytical techniques. Results revealed a total bacterial count of 1.34×10^5 cfu/ ml. Identity of bacterial genera isolated from wastewater were *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* (Gram negative) while the Gram positive bacteria were *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis* and *Lactobacillus* sp. Results also revealed that treatment of wastewater sample with EMF significantly reduced the bacterial load and physicochemical parameters of the sample and dependent on EMF intensity and duration of exposure. Results revealed a decline in bacterial population from 1.34×10^5 - 1.0×10^4 at intensities of 70nT-130nT and exposure time of 0- 144hr with the lowest count obtained at 130nT and the highest at 70nT. This study concludes that the use of Electromagnetic Field holds promise as an effective, non-invasive and eco-friendly strategy to improve the physical, chemical and biological quality of wastewater before discharge into the environment.

DOI: <https://dx.doi.org/10.4314/jasem.v26i4.9>

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Dates: Received: 13 February 2022; Revised: 13 March 2022; Accepted: 07 April 2022

Keywords: Bacteria, Bioremediation, Cassava wastewater, Environmental pollution

Water is vital to the survival of humans, animals, plants and all forms of life including microorganisms. There is no alternative to water in many of its daily uses and therefore must be sustained. Industries also depend on water for power generation and manufacturing processes and thus generate large quantity of wastewater during various manufacturing activities. Wastewater is any water that has been adversely affected in quality by anthropogenic influence which can serve as habitat for pathogenic microbes with serious implication on health of the populace. Wastewater can result from a combination of domestic, industrial, commercial or agricultural activities, surface runoff or storm water, and from sewer inflow or infiltration (Burton *et al.*, 2013) with industrial wastewaters being the most important sources of toxic contaminants in any environment (Murugesan, 2018). Rapid industrialization and

urbanization have enhanced the levels of organic pollutants in the environment. Due to increase in industrial activities, the ground water and surface waters are frequently polluted to such extent that it deteriorates in quality due to the presence of countless impurities and microorganisms and hence cannot be used for domestic purposes without treatment. The threat to human and aquatic lives posed by industrial liquid and gaseous effluents is enormous and burdensome as a consequence of the discharge of toxicants and potential pathogens into the environment. Water becomes unsuitable for human consumption or domestic usage when contaminated because it may be injurious to human health (Seema, 2015). Contaminated water can destroy aquatic lives and reduce their reproductive ability (Divya *et al.*, 2015). Environmental problems and potential hazards caused by industrial wastewater have prompted many

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countries to limit the discharge of polluting wastewater in receiving waters (Seema, 2015). Okafor, (2011) reported that ten percent of world's rivers are heavily polluted with effluent discharges from food and allied industries and users of these rivers and streams are constantly exposed to health-related risks due to indiscriminate discharge of industrial wastewater. The increased importance of agricultural products processing, chemical and food industries in economic development as well as in food security particularly in Nigeria demands that processing and waste handling should be given more attention (Enerijiofi, 2017). Cassava (*Manihot esculanta* Crantz) is a root tuber crop that is widely cultivated in tropical regions and processed to meet local food demands. Cassava peels and the liquid squeezed out of the mash and washing water are among the different wastes generated during the processing of cassava. The indiscriminate discharge of cassava wastewater contributes to environmental pollution and when discharged into water bodies cause contamination of rivers and lakes, thereby constituting health risk by introducing diverse microorganisms into the streams and alteration of the physicochemical parameters of streams and lakes. Different microorganisms have been isolated from industrial effluents. The bacteria compositions of wastewater may however vary as shown by other studies wherein *Pseudomonas*, *Acinetobacter* and *Enterobacteriaceae* have been observed to be the dominant bacteria present in municipal sewage water (Dubey *et al.*, 2011; Shah, 2017). Microorganisms such as *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Acinetobacter* and *Zooglea* sp have been isolated from sludge treatment of municipal wastewater (Divya *et al.*, 2015; Shah, 2017). Sugar mill effluents were reported to have *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Enterobacter aeruginosa* and *Escherichia coli* as the major bacterial groups (Buvaneswari *et al.*, 2013). Also, *Bacillus* sp, *Bacillus subtilis*, *Lactobacillus acidophilus*, *Aerococcus viridens* and *Corynebacterium manihot* were isolated from cassava wastewater by Arotupin (2007). *Bacillus megaterium*, *Bacillus subtilis*, *Alcaligenes faecalis*, *Pseudomonas* sp. and *Zooglea remigera* isolated from textiles waste water while species of *Pseudomonas stutzeri* and *Pseudomonas putida* were from domestic and other industrial waste waters (Baldwin *et al.*, 2008; Anitha and Eswari, 2012). According to Arotupin (2007) liquid wastewater generated during the processing of cassava tuber into various products has been reported to cause serious havoc to vegetation, houses and brings about infection. Public health and environmental sustainability concerns demand efficient wastewater management strategies in order to protect the

environment from deterioration and ameliorate the health burden imposed on the populace by untreated or improperly disposed wastewaters. Several methods have been used for the treatment of wastewaters before discharge, but more often the process again produces secondary contamination, which again re-contaminates the environment (Shah, 2017). This menace could be salvaged through biological cleaning which is a system that has a variety of applications such as decontamination of contaminated areas such as water, soil, sludge and flows. This biological approach also called bioremediation is lucrative, environmentally friendly and leads to the degradation of pollutants. Notwithstanding, after this effective method has been used, the challenge remains how to eliminate the microorganisms used for the remediation or treatment before discharge into environment. This is usually accompanied with chemical treatment such as disinfectant or application of heat which may be laborious and costly (Seema, 2015; Choudhari and Patel, 2018). The main drawbacks in bioremediation can be curtailed by the use of Electromagnetic Field (EMF) (Yadollahpour *et al.*, 2014; Choudhari and Patel, 2018). During the recent decades, electromagnetic fields (EMFs) have shown great potentials in environmental applications. Findings of the previous studies have demonstrated the high efficacy of EMFs as adjunctive or alternative of conventional wastewater treatment. EMFs can alter physical and chemical properties of water molecules, microorganisms and organic compounds and helps in prevention of scale formation in pipes in the treatment facilities, hence its suitability for wastewater treatment (Yadollahpour *et al.*, 2014; Adetuyi *et al.*, 2017; Beretta *et al.*, 2019). EMF is non-invasive, inexpensive, effective and environmentally friendly compared to the conventional method for water and wastewater treatments (Yadollahpour *et al.*, 2014; Adetuyi *et al.*, 2017; Beretta *et al.*, 2019). In addition, EMF has antimicrobial effect on the wastewater. Exposure of bacterial cell to EMF could cause a significant decrease or increase in the cell number. This decrease in cell number is as a result of EMF ability to induce biological effects on the bacterial cell (Gu *et al.*, 2012). Bayatiani *et al.*, (2019) stated that the biological effects on the bacterial cell could be positive, that is causing cell increase or negative that is causing cell death depending on some parameters such as frequency and intensity of the field, time of exposure and type of bacteria cells (Gram positive or negative). The objective of this study is therefore to assess the impact of electromagnetic field (EMF) on bacterial population and physicochemical properties of cassava wastewater.

MATERIALS AND METHODS

Sample collection: Cassava wastewater sample was collected at 10:00 am from a small-scale cassava industry located at Shagari village, along Akure - Benin expressway, Ondo State, Nigeria when it was freshly discharged at the processing factories. Samples for physicochemical analysis were put into one (1) liter plastic container while samples for microbiological analysis were taken with sterile sample bottles and transported in ice chest to the laboratory for analysis. Samples were collected in triplicates and were processed within forty-eight (48) hours of collection.

Microbiological analysis of the wastewater sample

Sterilization of glass wares and other equipment: All the glassware (Petri dishes, beakers, conical flasks) used were thoroughly washed with detergent and properly rinsed with tap water. They were air-dried and then sterilized in the hot air oven at 160°C for 2 h. The work bench was disinfected by swabbing with 70% ethanol.

Preparation of culture media and test solutions:

Culture media and test solutions were prepared according to manufacturer's specification in a conical flask, plugged with cotton wool and wrapped with aluminum foil after mixing the content and then heated to dissolve properly. All media were autoclaved at 121°C for 15 minutes holding time and allowed to cool to 45°C and aseptically poured into sterile Petri dishes and then left at room temperature (28.0 ± 2.0°C) to solidify.

Enumeration of bacterial population:

Serial dilutions were carried out on the cassava wastewater sample. 1.0 ml of wastewater sample was taken into 9 ml sterilized water in a test tube to form a stock solution up to 7th dilution and were cultured using the pour plate method on Nutrient Agar (NA) and then incubated at 35°C for 24 hours for the enumeration of total heterotrophic bacteria. The colonies which developed on the plates were counts in which the number of colonies were from 30-300 (Kumarasamy *et al.*, 2009) and its triplicate for each sample was selected. The average count was then multiplied by the dilution factor at that dilution and expressed as colony forming unit (CFU/ml) per milliliter of sample.

Characterization and identification of bacterial isolates:

Pure cultures of the isolates were obtained by repeated sub-culturing on nutrient agar plates. The isolates were characterized and identified using colonial and cellular morphology as well as biochemical reactions using Holt *et al.* (1994) and

Chessbrough, (2006) as standard references. Different tests carried out on pure isolates include; Gram reaction, spore staining, motility, catalase, oxidase, coagulase, nitrate reduction, methyl red, Voges-Proskauer, H₂S gas production, urease, citrate utilization, and sugar fermentation.

Physicochemical analysis of effluent sample: The physicochemical parameters analyzed in this study were temperature (°C), pH, electrical conductivity (µS/cm), turbidity (NTU), dissolved oxygen (mg/L), total solids (mg/L), total dissolved solids (mg/L), phosphate (mg/L), Sulphate (mg/l), Nitrate (mg/L), Potassium (mg/L), total hardness (mg/L), biochemical oxygen demand (BOD mg/L), Chemical oxygen demand (COD mg/L) and chloride (mg/L). These parameters were determined using the standard methods described by (Ademoroti, 1996; APHA, 2005).

Determination of the effects of EMF on bacterial load of wastewater:

An electric circuit (plate 1) that generated the electromagnetic field wave used for this research work was designed by the expert following the method of Adetuyi *et al.* (2017). The electromagnetic field pulse was generated from solenoid coil of hundreds of turns of copper wire. The coil was connected across a voltage source which induced magnetic field around the coil. 200 ml of wastewater in a conical flask was treated with EMF at varying intensities of 70nT, 80nT, 100nT, 110nT and 130nT and sampling was done before exposure (0 hr) and twelve (12) hour interval until 144 hrs for the enumeration of bacterial load and analysis of physicochemical properties.

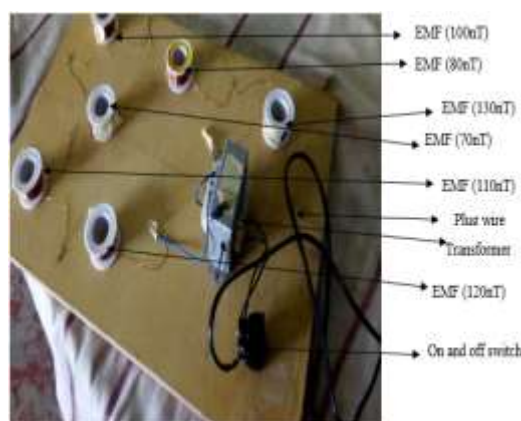


Plate 1: Electromagnetic field (EMF) apparatus setup

Statistical analysis: 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 were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Enumeration of bacterial population of the fresh raw cassava wastewater showed 1.34×10^5 cfu/ml. Table 1 revealed the morphological and biochemical characteristics of the bacterial isolates obtained from cassava wastewater sample. The wastewater consists of both Gram negative and Gram-positive bacteria. The Gram-negative bacterial isolates include *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi* while the Gram-positive bacteria were *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Lactobacillus* sp and *Micrococcus* sp. These organisms belong to different families consisting of Enterobacteriaceae, Micrococcaceae, Pseudomonadaceae, Bacillaceae and Lactobacillaceae.

Figure 1 shows the effect of EMF on bacterial population of cassava wastewater sample. Results revealed a decline in bacterial population from $1.34 \times$

10^5 - 1.0×10^4 (CFU/ml) at intensities of 70nT-130nT and exposure time of 0- 144 hr. The lowest count was obtained at 130nT and the highest at 70nT. The counts were 3.1×10^4 (CFU/ml), 2.5×10^4 (CFU/ml), 2.1×10^4 (CFU/ml), 1.8×10^4 (CFU/ml), 1.4×10^4 (CFU/ml) and 1.0×10^4 (CFU/ml) respectively at 70nT, 80nT, 100nT, 110nT and 130nT intensities at 144 hr of exposure. Table 2 and Figure 2 show the effect of EMF on the physicochemical characteristics of cassava wastewater. Results revealed a corresponding decrease in all the physicochemical characteristics of cassava wastewater with applied treatments. The values of physicochemical parameters of the wastewater sample were temperature ($27 - 25^\circ\text{C}$), turbidity (13.54 - 6.98), electrical conductivity (258 - 79 $\mu\text{S/cm}$), pH (6.32 - 5.78), 63-28 for chloride, Total hardness (210-78 mg/L), Sulphate (64 - 41mg/L), 39-21 (mg/L) for nitrate, phosphate (37-69.54 mg/L), TDS (186-52 mg/L), potassium decreased from 26.89 - 8.34 (mg/L), while DO, BOD and COD decreased from 56.23 (mg/L), 18.67 (mg/L), and 58.34 (mg/L) to 12.57 (mg/L), 13.56 (mg/L), and 11.21 mg/L respectively after exposure of 144 hrs at 130 nT.

Table 1: Morphological and biochemical characteristics of bacteria isolated from cassava effluent.

Characteristics	Waste water isolates								
	EI ₁	EI ₂	EI ₃	EI ₄	EI ₅	EI ₆	EI ₇	EI ₈	EI ₉
<i>Morphology</i>									
Cell Shape	Rod	Cocci	Rod	Rod	Rod	Rod	Rod	Cocci	Rod
Gram stain	-	+	+	-	-	-	+	+	+
Motility	+	-	+	+	+	+	-	-	+
Spore stain	-	-	+	-	-	-	-	-	+
<i>Biochemical characteristics</i>									
Catalase	+	+	+	+	+	+	-	+	+
Coagulase	-	+	-	-	-	-	-	-	-
Oxidase	-	-	-	-	+	-	-	+	+
H ₂ S Production	-	-	-	-	-	+	-	+	+
Citrate	+	+	+	-	+	-	-	+	+
Urease	-	+	-	-	-	-	-	-	-
VP	+	+	+	-	-	-	-	+	+
MR	-	+	-	+	-	+	-	+	-
Nitrate Reduction	+	+	-	+	+	+	-	+	+
<i>Fermentation of</i>									
Glucose	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	-	-	-	+	+	+
Maltose	+	+	+	+	-	+	+	+	+
Lactose	+	+	-	+	-	-	+	-	+
Mannitol	+	+	-	+	+	-	-	-	+
Possible	<i>E</i>	<i>Staph.</i>	<i>Bacillus</i>	<i>E. coli</i>	<i>Ps.</i>	<i>Sal.</i>	<i>Lactobacillus lactis</i>	<i>Micrococcus</i>	<i>Bacillus</i>
Organism	<i>aerogenes</i>	<i>aureus</i>	<i>cereus</i>		<i>aeruginosa</i>	<i>typhi</i>		sp	<i>subtilis</i>

Legend: EI₁ – EI₉ = Effluent isolates 1-9, + = Positive; - = Negative

The chemical, physical, biological and radiological characteristics of water defines its quality (Diersing, 2009). The quality of water is of vital concern to mankind since it is directly linked with human wellbeing and survival. Ranjana (2010) stated that groundwater quality has significant impact on the quality of public health. It is a measure of the condition of water relative to the need or purpose of living

organisms including humans. Wastewater cannot be used for domestic purposes without treatment due to the presence of countless impurities and microorganisms. In this study, the effect of EMF treatment on the bacteriological and physicochemical parameters of cassava wastewater was evaluated as a cheap, non-invasive approach to wastewater treatment before discharge unto water bodies or environment to

mitigate its consequences on human health and the environment. The results from this study suggest that the bacterial community of cassava wastewater is diverse. Heavy load of bacteria of diverse genera was obtained from the cassava wastewater. Although, the population of bacteria obtained in this study was less than that obtained by Arotupin (2007), but the type and kind of bacteria were among those isolated in his study of cassava wastewater.

The high microbial count may be attributed to lack of efficient control measures in the processing of cassava. The high load of bacteria could be due to fermentable carbohydrate and other mineral elements in the wastewater which provide nutrients and support the growth and cellular activities of the microbes. This assertion is in line with the report of Oboh and Akindahunsi (2003) who documented that cassava wastewater contains cyanogenic glycosides and essential elements such as Zn, Mg Fe, Ca, Na and K. The sources of the different microorganisms could be from the cassava tuber, soil, washing water, processing equipment and handlers. The presence of *Salmonella typhi* and *Escherichia coli* indicates coliform presence of which *E. coli* is an indicator organism for fecal contamination and hence a potential source for the transmission of diarrhea and dysentery and other diseases of public health concern. Different methods have been employed in the treatment of wastewater and each method has its advantages and disadvantages. According to Yadollahpour *et al.* (2014) and Choudhari and Patel, (2018) the challenge of how to eliminate the microorganisms used for the remediation or treatment before discharge into environment can be curtailed by the use of Electromagnetic Field (EMF). In this study, exposure of cassava wastewater sample to EMF caused a significant decrease in the microbial population and the physicochemical properties of the sample but vary with frequency, intensity of the field and duration of exposure. Gu *et al.* (2012) asserted that the decrease in cell number is as a result of EMF ability to induce biological effects on the bacterial cell. Result therefore suggests that EMF has antibacterial effect on the isolates from wastewater.

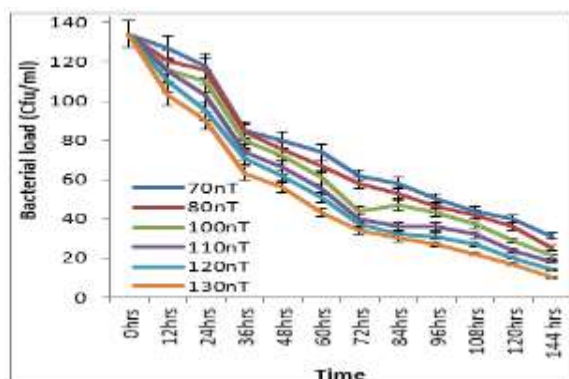


Fig 1: Effect of EMF at different intensities and time intervals on bacterial loads of wastewater from Cassava (Error bar ±1 SE)

Table 2: Effect of EMF (130nT) treatment on the physical characteristics of cassava wastewater at 144 hr exposure time.

Parameter	Raw cassava waste water	Treated cassava waste water
Temperature (°C)	27.01 ± 0.0	25.0 ± 0.0
pH	6.33 ± 0.0	5.98 ± 0.0
Turbidity (NTU)	13.55 ± 0.01	6.98 ± 0.0
Electrical Conductivity (µS/cm)	258.03 ± 0.05	79.1 ± 0.08
TS (mg/L)	210.13 ± 0.05	69.58 ± 0.0
TDS (mg/L)	186.03 ± 0.05	52.13 ± 0.05

Note: Values are expressed mean ± SD

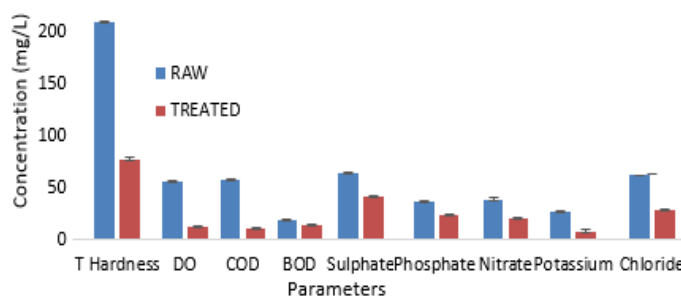


Fig 2: Effect of EMF (130nT) treatment on the chemical characteristics of cassava wastewater at 144 hr exposure time.

*Significant $p < 0.05$.

This finding corroborates the assertion of Moulder (2002) that the pulsed electric field treatment enhanced bactericidal action and hence EMF treatment significantly reduced the growth and proliferation of bacteria. The lowest EMF strength (70nT) had the least effect on the bacterial population while the highest effect was obtained with the highest

EMF intensity of 130nT. This result agrees with the report of Adetuyi *et al.*, (2017) and hence suggests that the higher the EMF intensity, the greater the bactericidal action. According to Adetuyi *et al.* (2017), one of the probable explanations of EMF on the bacterial population might be due to denaturing effect of EMF on metabolites of microbial activity or the

rotating electric field formed by the variable magnetic field. Water resources pollution and the respective groundwater quality degradation are caused by diverse human activities which result in the change of water physicochemical characteristics. In this study, evaluation of the impact of EMF treatment of cassava wastewater on the physicochemical parameters revealed that all the parameters were significantly reduced suggesting the effectiveness of EMF in the restoration of water quality. pH is an indicator of acidic or alkaline condition of any medium as ranked on a scale of 0 to 14. The lower the pH, the more acidic it is and vice versa. pH affects many chemical and biological processes in water and different organisms have different ranges of pH within which they flourish. The pH (6.32-5.78) of the raw and treated wastewater were below 6.5- 8.5 WHO permissible standard for portable water. The decrease in pH of sample can cause the drastic fall in bacterial population as bacterial thrive better in near alkaline pH than acidic medium. This decrease could be due to acidic metabolites produced during microbial activities. Turbidity may be due to suspended solids and colloidal matter in the grinded cassava tuber or processing water. It may also be due to the growth of micro-organisms resulting in increase of cell number, if not of bacteria but of other organisms forming the microbial community. Turbidity of the effluent decreased from 13.54 to 6.98 NTU. This reduction in turbidity probably implies that microbial growth and activity have been hindered upon exposure to varying intensities of EMF, hence the reduction in bacterial load which implies the effectiveness of treatment. The presence of chloride in water can be attributed to dissolution of salt deposits being part of various chemicals contaminating agricultural soil, processing water and handlers, thereby leading to accumulation of chloride in tuber and wastewater. Total hardness is the traditional measure of the capacity of water to precipitate soap. Biological oxygen demand denotes the amount of dissolved oxygen needed by aerobic biological organisms to break down organic material present in a given water sample at certain temperature over a specified period of time. BOD reduction is used as a gauge of the effectiveness of wastewater treatment. Treatment of effluent with EMF results in the reduction of BOD and this suggests reduction in dissolved oxygen (DO) for use by aerobic microorganisms for the metabolism of organic matter in effluent and may consequently lead to cell death and reduction in population. The same applies to the chemical oxygen demand (COD). Nitrogen, Phosphorous, Sulphur and Potassium are among essential mineral elements for microbial growth. The inadequacy or lack of these mineral elements will adversely affect cell structure and metabolism. The

presence of these elements could be of both organic and inorganic sources. In cassava effluent, these elements could come with the cassava tuber from NPK fertilizer applied to soil, processing water or other chemicals. Reduction of these mineral elements probably indicates their utilization by microbes for cellular activities.

Conclusion: Environmental problems and potential hazards occasioned by cassava wastewater can be reduced by treatment with EMF before wastewater discharge into receiving waters or environment. This method of wastewater treatment is non – invasive, environmentally friendly and does not produce known secondary contamination or adverse environmental consequences. This study concludes that EMF has good bactericidal potential, modifying the physicochemical properties of the wastewater thereby reducing risk of infection with potential pathogens as well as chemical toxicants if wastewater is discharged into the environment.

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