



## **Lf magnetic field effects on microbial species involved in fermentation of cereals for bioethanol production**

**<sup>1</sup>A.O. AJAYI, <sup>2</sup>C.T. ODELUYI and <sup>3</sup>N.O. AJAYI**

<sup>1,2</sup> Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria

<sup>3</sup> Department of Physics and Electronics, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria

\*Corresponding author E-mail address: [olajide.ajayi@aaau.edu.ng](mailto:olajide.ajayi@aaau.edu.ng), [jidet02@yahoo.com](mailto:jidet02@yahoo.com)

### **Abstract**

#### **Keywords:**

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*This study shows the efficacy of electromagnetic radiation on microbes during fermentation and storage of grains for the possibility of improving the life span of cereals meant for bioprocessing, consumption and food security. Low frequency Lf magnetic radiation tested at 1000mG and 5000mG also enhances the antimicrobial susceptibility pattern of some resistance strains on exposure to radiation. The magnetic radiation also enhances the antimicrobial susceptibility pattern of some resistance strains on exposure to radiation. Most of the organisms are susceptible particularly to imipenem (IPM), followed by vancomycin (VA) and piperacillin (PRL). But other antibiotics tested are less effective. The exposure of the isolates to Lf magnetic field of 1000mG and 5000mG show a decrease in microbial growth rate whereby isolate MD<sub>1</sub> that had  $29 \times 10^3$  cfu/ml in 15 minutes exposure reduced to  $9 \times 10^3$  cfu/ml after one hour of exposure. Similarly, MD<sub>1</sub> that had  $27 \times 10^3$  cfu/ml in 15 minutes exposure reduced to  $6 \times 10^3$  cfu/ml after one (1) hour of exposure. Similar pattern of radiation effect and growth rate were recorded for other isolate exposed in this way. The result obtained in this study shows that resistant organisms could be susceptible to antibiotics by exposure to magnetic radiation which could be a solution to antimicrobial resistant strains that may cause food spoilage. This can also be helpful in storage facilities to improve shelve life of food.*

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### **INTRODUCTION**

The electromagnetic (EM) waves that compose electromagnetic radiation can be imagined as a self-propagating transverse oscillating wave of electric and magnetic fields. Electromagnetic radiation (EMR) refers to the waves (or their quanta, photons) of the electromagnetic field, propagating (radiating) through space-time, carrying electromagnetic radiant energy (Purcell and Morin, 2013). It includes radio waves, microwaves, infrared, (visible) light, ultraviolet, X-rays, and gamma rays (Browne and Michael, 2013). Classically, electromagnetic radiation consists of electromagnetic waves, which are synchronized oscillations of electric and magnetic fields that propagate at the speed of light through a vacuum. The oscillations of the two fields are perpendicular

to each other and perpendicular to the direction of energy and wave propagation, forming a transverse wave. The wave front of electromagnetic waves emitted from a point source (such as a light bulb) is a sphere. The position of an electromagnetic wave within the electromagnetic spectrum could be characterized by either its frequency of oscillation or its wavelength. The electromagnetic spectrum includes, in order of increasing frequency and decreasing wavelength: radio waves, microwaves, infrared radiation, visible light, ultraviolet radiation, X-rays and gamma rays (Maxwell and Clerk, 2017).

Electromagnetic waves are produced whenever charged particles are accelerated, and these waves can subsequently interact with other charged particles. EM waves carry energy, momentum and

angular momentum away from their source particle and can impart those quantities to matter with which they interact. Electromagnetic radiation is associated with those EM waves that are free to propagate themselves ("radiate") without the continuing influence of the moving charges that produced them, because they have achieved sufficient distance from those charges. Thus, EM radiation is sometimes referred to as the far field. In this research, the near field refers to EM fields near the charges and current that directly produced them.

In the quantum theory of electromagnetism (Maxwell and Clerk, 2017), EMR consists of photons, the elementary particles responsible for all electromagnetic interactions. Quantum effects provide additional sources of EMR, such as the transition of electrons to lower energy levels in an atom and black-body radiation. The energy of an individual photon is quantized and is greater for photons of higher frequency. The effects of EMR upon chemical compounds and biological organisms depend both upon the radiation's power and its frequency. EMR of visible or lower frequencies (i.e., visible light, infrared, microwaves, and radio waves) is called non-ionizing radiation, because its photons do not individually have enough energy to ionize atoms or molecules. The effects of these radiations on chemical systems and living tissue are caused primarily by heating effects from the combined energy transfer of many photons. In contrast, high ultraviolet, X-rays and gamma rays are called ionizing radiation since individual photons of such high frequency have enough energy to ionize molecules or break chemical bonds. These radiations have the ability to cause chemical reactions and damage living cells beyond that resulting from simple heating, and can be a health hazard (Balmori, 2016).

Bio-electromagnetics is the study of the interactions and effects of EM radiation on living organisms (Oncul *et al.*, 2016). The effects of electromagnetic radiation upon living cells, including those in humans, depend upon the radiation's power and frequency. For low-frequency radiation (radio waves to visible light) the best-understood effects are those due to radiation power alone, acting through heating when radiation is absorbed. For these thermal effects, frequency is important only as it affects penetration into the organism (for example, microwaves penetrate better than infrared). It is widely accepted that low frequency fields that are too weak to cause significant heating could not possibly have any biological effect (Binhi and Vladimir 2017).

The reactive ions are produced in the foods due to irradiation that destroys that microorganism

immediately by changing the structure of cell membranes and affecting metabolic enzyme activity (Brooke *et al.*, 1966). However, a more important effect is on the DNA and RNA molecules in cell nucleus, which are required for the growth and replication. The effects of irradiation only become apparent after a period of time when the DNA double helix fails to unwind and the micro-organism cannot reproduce by cell division. The rate of destruction of individual cells depends on the rate at which ions are produced and inter react with DNA, where as the reduction in cell number depends upon the total dose of radiation received. Some bacterial species contain more than one molecules of DNA and others are capable of repairing damaged DNA and in such cases we find that the rate of destruction is not linear with received dose (given growth) (Gould, 1991). This study therefore aims at determining the potential of electromagnetic radiation on microbes during fermentation and storage of grains for the possibility of improving the life span of cereals meant for bioprocessing, consumption and food security.

## MATERIALS AND METHODS

### Sample source and materials used

Three different low-grade grains (corn, millet and sorghum) were bought from Ibaka Market, Akungba-Akoko. These were used separately for Ethanol production. Akungba Akoko is located in Akoko South West Local Government, Ondo State, Nigeria Within longitude 5.44°E and 5.45° E and latitude 7.24N and 7.28N (Ologunbode, 2003). Materials and culture media such as nutrient Agar, Eosin Methylene Blue agar (EMB) and Potato dextrose agar used for this study were adequately sterilized.

### Procedure for fermentation

Corn, millet and sorghum were washed in sterile distilled water. Three hundred grams of the grains were washed and placed in a clean bowl containing 2 litres of distilled water separately and then grinded. The grinded mash was allowed to ferment for 3 days at room temperature  $30 \pm 2^{\circ}\text{C}$  (Ojokoh, 2007).

### Antibiotic sensitivity testing

Antibiotics susceptibility test was performed on bacterial isolates cultured on Mueller-Hinton agar using Kirby-bauer disk diffusion method (CLSI, 2011). A growth medium, usually Mueller-Hinton agar, is first evenly seeded throughout the plate with the isolate of interest that has been diluted at a standard concentration. Commercially prepared disks, each of which is pre-impregnated with a standard concentration of a particular antibiotic, are then evenly dispensed and lightly pressed onto the

agar surface. The test antibiotic immediately begins to diffuse outward from the disks, forming a concentration gradient around each disk. After an overnight incubation, the bacterial growth around each disc is observed. If the test isolate is susceptible to a particular antibiotic, a clear area of no growth will be observed around that particular disk. This zone will be measured in mm and compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible or resistant.

#### Treatment of isolates with If magnetic fields.

This test was conducted in the Department of Physics, Adekunle Ajasin University, Akungba Akoko, where a solenoid coil to produce an Lf magnetic field existed. Isolates from the stock culture were transferred unto freshly prepared plates and afterwards multiplied in liquid nutrient medium (broth). From every breed, 1ml inoculums were introduced into 9ml freshly prepared broth in test tubes. The test tubes were treated with If magnetic field of 1000mG and 5000mG for 15, 30, 45 and 60 minutes. The untreated isolates were taken as control. After the treatment with magnetic field the isolates were seeded on a Petri dish containing selected medium, incubated at 37°C for 24 hours and 25°C for 3-5days for both bacteria and fungi respectively. The same process was used for the untreated ones. The colony count was applied for bacteria and the spore counts were applied for the fungi to determine the effect of magnetic field using colony counter.

#### RESULT

This study shows the efficacy of electromagnetic radiation on microbes during food fermentation processes and food safety. The electromagnetic rays also enhance the antimicrobial susceptibility pattern of some resistance strains on exposure to radiation as shown in Table 1. Most of the organisms are susceptible particularly to imipenem (IPM), followed by vancomycin (VA) and piperacillin (PRL). But other antibiotics tested are less effective (Tables 1 and 2).

The effect of electromagnetic field (EMF) radiation on exposed microbial isolates at 1000mG shows reduction of microbial load in 15 mins of exposure from  $2.9 \times 10^4$  cfu/ml to  $9.0 \times 10^3$  cfu/ml after one (1) hour compared with the control which is  $4.4 \times 10^4$  cfu/ml for isolate MD<sub>1</sub>. Similar pattern of radiation effect and growth range were obtained for other isolates exposed in this way. Furthermore, more reduction of microbial load was observed using higher radiation field of 5000mG whereby isolate MD<sub>1</sub> had  $2.7 \times 10^4$ cfu/ml in 15 mins exposure reduced to  $6.0 \times 10^3$ cfu/ml after one (1) hour of exposure (Table 3). Similar observations were made for other isolates up to SD<sub>4</sub> (Table 4).

**Table 1: Antibiotics susceptibility test (in mm) after exposure to EMF for 60mins**

Isolate code	V A	IP M	O B	O X	FO X	PR L	SF T
EMB	-	16	10	-	-	-	-
CD <sub>3</sub>							
PDA	-	24	-	-	-	18	-
CD <sub>4</sub>							
NACD <sub>3</sub> <sup>i</sup>	-	10	-	-	-	-	-
NASD <sub>3</sub> <sup>i</sup>	-	20	-	-	-	-	-
NACD <sub>1</sub>	-	20	-	-	-	-	-
PDAP	-	5	-	-	-	-	-
SD <sub>4</sub> <sup>i</sup>							
NA SD <sub>2</sub> <sup>i</sup>	-	22	-	-	-	-	-
NA	-	-	-	-	-	16	-
MD <sub>3</sub> <sup>i</sup>							
NA	-	20	-	-	-	-	-
CD <sub>2</sub> <sup>i</sup>							
EMBC	-	12	-	-	-	-	-
D <sub>2</sub> <sup>iii</sup>							
EMBM	-	10	-	-	-	-	-
D <sub>3</sub> <sup>ii</sup>							
PDA	-	-	-	-	-	-	-
MD <sub>3</sub> <sup>i</sup>							
U <sub>3</sub>	-	18	-	-	-	-	-
EMBM	18	24	12	-	-	-	-
D <sub>3</sub> <sup>i</sup>							
PDA	-	20	-	-	-	-	-
CD <sub>1</sub>							
NA SD <sub>1</sub>	-	30	-	-	-	-	-
NA	-	20	-	-	-	-	-
MD <sub>1</sub>							
EMBSD	-	16	-	-	-	-	-
<sup>3</sup> EMBSD	-	14	-	-	-	-	-
<sup>3</sup> EMBC	-	18	14	-	-	15	-
D <sub>1</sub>							

**KEY**  
 VA — Vancomycin, IPM — Imipenem  
 OB — Ofloxacin, OX — Oxacilin  
 FOX — Cefocitin, PRL — Piperacilin  
 SFT — Co-trimaxole

**Table 2: Antibiotics susceptibility test after exposure to EMF for 30mins (in mm)**

Isolate code	V A	IP M	O B	O X	FO X	PR L	SF T
NASD <sub>3</sub>	-	8	12	-	-	-	-
PDA	-	20	14	-	-	10	-
MD <sub>3</sub>							
NASD <sub>2</sub>	-	30	-	-	-	14	-
EMBS	-	20	-	-	-	-	-
D <sub>4</sub> <sup>i</sup>							
PDAC	-	32	20	-	-	-	-
D <sub>1</sub>							

NACD <sub>1</sub>	-	26	-	-	-	-	-
EBM	-	20	18	-	-	-	-
SD <sub>3</sub> <sup>i</sup>							
EMB	20	-	-	-	-	14	-
MD <sub>3</sub> <sup>i</sup>							
NA	-	-	16	-	-	-	-
CD <sub>2</sub>							
PDA	-	15	-	-	-	-	-
CD <sub>4</sub>							
NA	-	-	-	-	-	-	-
SD <sub>1</sub>							
EMB	-	22	-	-	-	-	-
CD <sub>2</sub>							
EMB	-	20	16	-	14	-	-
CD <sub>1</sub>							
EMB	-	22	-	-	-	-	-
MD <sub>2</sub>							
U <sub>3</sub>	-	20	-	-	-	-	-
NA	-	26	10	-	-	-	-
MD <sub>1</sub>							
NA	-	-	-	-	10	-	-
CD <sub>3</sub>							
NA	-	16	-	-	-	12	-
MD <sub>3</sub>							
X	-	24	-	-	-	-	-

Key: VA= Vancomycin, IPM= Imipenem, OB= Ofloxacin, OX= Oxacillin  
 FOX= Cefocitin PRL= Piperacillin  
 SFT=Co-trimoxazole.

### DISCUSSION

The use of electromagnetic radiation can control microbes during fermentation and has the potential to improve cereal food storage facilities as intensified in this study (Tables 1 and 2). The exposure of the isolates to Lf magnetic field of 1000mG and 5000mG show a decrease in microbial growth rate. In this study isolate MD<sub>1</sub> had population growth of 29x10<sup>3</sup> cfu/mL in 15 minutes exposure reduced to 9x10<sup>3</sup> cfu/mL after one hour of exposure. (Table 3) similarly, MD<sub>1</sub> also had 27x10<sup>3</sup> cfu/mL in 15 minutes exposure reduced to 6x10<sup>3</sup> cfu/mL after one (1) hour of exposure. (Table 4).

**Table 3: Microbial load (in cfu/ml × 10<sup>-3</sup>) after exposure to EMF at 1,000mG**

Isolate code	Time of Exposure (mins)				
	15mins	30mins	45mins	60mins	Control
MD <sub>1</sub>	29	14	31	09	44
MD <sub>2</sub>	33	30	22	05	TNC
MD <sub>3</sub>	26	09	63	24	56
MD <sub>4</sub>	TNC	121	89	71	TNC
CD <sub>1</sub>	90	41	33	32	106
CD <sub>2</sub>	TNC	102	91	18	TNC
CD <sub>3</sub>	57	61	108	90	TNC
CD <sub>4</sub>	TNC	72	64	54	76
SD <sub>1</sub>	54	22	47	42	91
SD <sub>2</sub>	68	40	48	21	63
SD <sub>3</sub>	12	09	17	03	25
SD <sub>4</sub>	53	41	23	18	37

Key: MD<sub>1</sub>= millet day 1, MD<sub>2</sub>= millet day 2, MD<sub>3</sub>= millet day 3, MD<sub>4</sub>=millet day 4, CD<sub>1</sub>=Corn day 1, CD<sub>2</sub>=Corn day 2, CD<sub>3</sub>=Corn day 3, CD<sub>4</sub>=Corn day 4, SD<sub>1</sub>=Sorghum day 1, SD<sub>2</sub>=Sorghum day 2, SD<sub>3</sub>=Sorghum day 3, SD<sub>4</sub>=Sorghum day 4.

**Table 4: Microbial load (in cfu/ml × 10<sup>-3</sup>) after exposure to EMF at 5,000mG**

Isolate code	TIME OF EXPOSURE(mins)(in cfu/ml × 10 <sup>-3</sup> )				
	15mi n	30mi n	45mi n	60mi n	Contr ol
MD <sub>1</sub>	27	18	26	06	44
MD <sub>2</sub>	168	36	52	61	TNC
MD <sub>3</sub>	19	08	44	11	56
MD <sub>4</sub>	96	53	48	28	TNC
CD <sub>1</sub>	94	52	31	30	106
CD <sub>2</sub>	112	76	40	21	TNC
CD <sub>3</sub>	57	51	70	15	TNC
CD <sub>4</sub>	70	42	22	05	76
SD <sub>1</sub>	60	53	15	13	91
SD <sub>2</sub>	30	27	17	11	63
SD <sub>3</sub>	21	20	09	03	25
SD <sub>4</sub>	18	26	11	07	37

Key: MD<sub>1</sub>= millet day 1, MD<sub>2</sub>= millet day 2, MD<sub>3</sub>= millet day 3, MD<sub>4</sub>=millet day 4, CD<sub>1</sub>=Corn day 1, CD<sub>2</sub>=Corn day 2, CD<sub>3</sub>=Corn day 3, CD<sub>4</sub>=Corn day 4, SD<sub>1</sub>=Sorghum day 1, SD<sub>2</sub>=Sorghum day 2, SD<sub>3</sub>=Sorghum day 3, SD<sub>4</sub>=Sorghum day 4.

This observation is consistent with the study of Saleem *et al.*, (2018a and 2018b) which shows that different magnetic fields affect the growth pattern of bacteria differently, depending on the bacterial strain. The weak magnetic field seems to decelerate the growth rate, even after the magnetic field is removed. Similar pattern of radiation effect and growth rate were recorded for other isolate exposed in this way. This finding also correlates with the study of (Lipic and Barabass, 2004) who investigated the effect of extremely low frequency on the growth rate of both Gram-positive and Gram-negative bacteria.

The results showed the effect of electromagnetic field at different intensities on microorganisms which reveal the initiation of some growth abilities in some microorganisms and it inhibit some essential particle in some microorganisms. This study shows that the electromagnetic field induces a decrease in growth rate of tested organisms at certain level of exposure with respect to control. This is in line with the work of Strasak, *et al.*, (2002). There was also an increase in growth of some organisms when they were exposed at a field of magnitude of 5000mG. This shows that a modification has occurred in the cell composition of the microorganisms. The results indicated that the studied microorganisms could switch on new pathways to adapt themselves against stress induced by exposure to electromagnetic field.

The isolates that are *resistant* to antibiotics are also exposed to Lf magnetic field for 30 and 60 minutes and were tested again for their sensitivity. It was observed at this stage that the organisms shows tangible susceptibility to the antibiotics used. Therefore, resistant organisms could be susceptible to antibiotics by exposure to electromagnetic radiation as reported by (Lipic and Barabass, 2004) who found out that the use of electromagnetic field could cause either a decrease or an increase in the sensitivity of exposed cells resulted in either inhibition or stimulation effects on organisms depending on the drug mode of action on the cells of the organisms. The influence of electromagnetic wave intensified in this study has gone a long way to show the usefulness of electromagnetic radiation for both storage of food stuff as well as reducing antibiotic resistant strains in our environment coupled few other advantages that can be explored in the nearest future.

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