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Original Article

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Survey of Magneto-tactic Properties of *Escherichia coli* Under Static Magnetic Fields

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Abstract
Some of the microorganisms such as *Escherichia coli* have the ability to migrate to areas in which the intensity of magnetic fields (MFs) is higher, which is called magnetotactic properties. Magnetotaxis is a process implemented by a group of gram-negative bacteria that involves orienting and coordinating movement in response to magnetic fields. This study was conducted to investigate these properties of *Escherichia coli* in laboratory conditions. By means of coated wires (30 rounds) placed in two parts of the reactor (with five zones and a volume of 250 mL) and direct current (DC), an intensity of 0.18 mT for 42 minutes has been prepared. The most probable number of *E. coli* per 100 mL (MPN/100 mL) in each zone of the reactor, before and after exposure, was estimated. According to the results of this study, *E. coli* has magnetotactic properties, and the mean density of these bacteria in higher MFs (0.18 mT) is higher compared to the other zones in the reactor.
Keywords: Magnetic fields, *Escherichia coli*, Magnetotactic properties

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1. Introduction

Magnetotactic bacteria (MTB) are Gram-negative bacteria that have the ability to align and navigate along the Earth's geomagnetic fields and some other external magnetic fields (MFs). They have intracellular chains of magnetic iron minerals, either magnetite (Fe_3O_4) or greigite (Fe_3S_4) (1).

MTB by rotating their helical flagella can swim in the water. *Escherichia coli* is one of the microorganisms with a larger size and less flagellar proteins (2). MTB has been found in the sediments and freshwater, brackish, marine, and hypersaline habitats (3). According to a study by Talib et al, MTB are found worldwide in aquatic environments such as freshwater and marine ecosystems (4). In the division of MTB based on whether or not it is aerobic or anaerobes, facultative anaerobes, or micro-aerobic. It should be mentioned that a few of them appear to be capable of growing under aerobic conditions (5).

Magnetotactic microorganisms possess flagella and are rich in iron, within intra-cytoplasmic membrane vesicles. These cellular structures impart a magnetic moment to the cells. This nono-engine (flagella) has the main roll of magnetotactic (6).

These observations highlight the interesting diversity of microbiological species. Generally, they swim to the magnetic north in the northern hemisphere, to the magnetic south in the southern hemisphere, and both ways on the geomagnetic equator (7).

In 1975, the first peer-reviewed article on MTB was published by Blakemore. He reported that the bacteria were capable of orienting themselves in a certain direction of MFs. He observed that these microorganisms followed the direction of Earth's MF (from south to north) and named them as MTB (8).

In 2006, Bellini mentioned that they have the capability of synthesizing unique intracellular organelles (the magnetosomes), that is single-domain magnetic crystals of magnetite or greigite, which are covered by biomembranes. Cytoskeleton MamK filaments enable the magnetosomes to be organized into chains (9). Magnetotactic bacteria have high numbers of chemotaxis transducers and proteins involved in cellular signaling and bacterial taxis which might be related to the control of magneto-taxis (10). Magnetosome chains impart a net magnetic dipole moment to the cell, which allows cells to align and swim along geomagnetic field lines (11).

Magneto-taxis behavior of some microorganisms facilitated the movement of them to locate at the preferable oxic anoxic interface in chemically stratified sediments or water columns (12). MTB needier on not only for their growth but also a production of their magnetotactic apparatus composed of mineralized iron. The accumulation of iron is 100 to 1000 times higher in MTB than in other microorganisms (13,14).

Amann et al presented various morphotypes of microorganisms including, vibrio, spirilla, cocci, rod-shaped, and more complex multicellular magneto-tactic prokaryotes that are called magneto-globules. It should be noted that Lei Yan classified microorganisms based on the effects of MFs on their behaviors, which include Magneto-spirillum, Magneto-vibrio, Magneto-coccus, and so on (15).

One of the well-known microorganism that has less flagellar proteins and a larger size is *Escherichia coli* (2). As mentioned by Letuta and Tikhonova, the ability of living organisms to respond to MF exposure has been repeatedly demonstrated experimentally. The correlation between the MFs (30, 60, 80, and 100 mT) and the growth rate of *E. coli* was reported (16). *E. coli* has many properties. It can be obtained easily and can be cultivated at a temperature of 37°C; therefore, it is a known bacterial strain in course of researches on MFs (14, 17). Based on the physiological functions of microorganisms, Dini and Abbro classified MFs into the following groups: weak (<0.001 T), moderate (0.001–1 T), strong (1–5 T), and ultra-strong (> 5 T) (18). Moderate SMF affected bacterial growth (19, 20). However, Ji et al showed that a SMF intensity of 450 mT during the 60 min exposure inhibited the bacterial growth and even killed *E. coli* (21).

In this study, we tried to survey magneto-tactic properties of *Escherichia coli* under static MFs in laboratory condition. It must be mentioned that the static MFs are constant fields, without any change in intensity or direction during the time of exposure, and they have a zero frequency (22).

2. Materials and Methods

2.1. Reactor or Experimental Device

The reactor used in this study was a cylinder (a batch reactor made of chromium and vanadium), with a length of 35.5 cm and thickness of 3 cm. There were 5 zones on one side of the cylinder.

Each area had a diameter of 2 cm, the distance between two zones was 2.5 cm, and the volume of the cylinder was 250 mL. At the beginning of each test, this device was sterilized by the oven (170°C for 1 hour). For better understanding the process and comparing the density of *E. coli* after each run of the test, the zones on the left (left zone) and right (right zone) of the cylinder were named as shown in Fig. 1.

2.2. The Intensity of Magnetic Fields

To generate uniform MFs, 30 turn coils were placed around the two parts of reactors. As the intensity of MFs produced in this study was low (0.18 mT), the use of two solenoids in the same area of the cylinder was considered. Copper coated wires with a thickness of 0.5 mm were used. They were fed by DC power supply (5A) (DAZHENG PS-305D) and plugged into a lamp as the consumer . The effective current was 4.90 A, which was measured using amperemeter. The intensity of MFs in this experiment was equal to 0.18 mT in the center of coils and the whole duration of the test was calculated by equation 1.

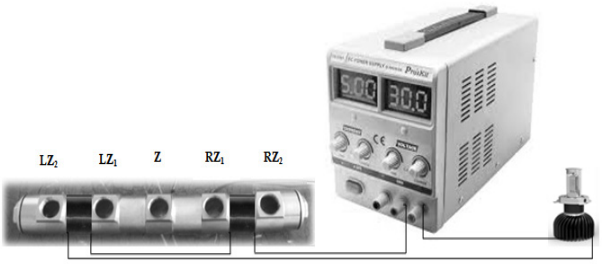

Fig. 1. Experimental Apparatus.

$$B = \mu NI \quad (1)$$

Where

B = intensity of magnetic fields (T)

μ = permeability of water (1.26×10^{-6} H/m)

N = number of turns

I = effectiveness of current (A)

Then

$B = 1.26 \times 10^{-6} \times 30 \times 4.9 = 0.00018$ T = 0.18 mT

The coil was placed in the middle of the cylinder to get a homogeneous and higher MF strength.

2.3. Medium Culture and Samples

Escherichia coli was cultured on the plate in the microbial the microbial lab. Non-pathogenic *E. coli* ATCC 25922 was used in this study. Using fildoplatin, one loop of a colony-forming *E. coli* on the plate was taken and added into 500 mL of distilled water as the main samples, and then the main sample was shaken well until the bacteria were distributed uniformly in water. Then, 250 mL of the main sample was poured into the reactor. In this part of the study, the samples in the reactor were called case and the residual solution in the main sample was called the control sample. An important point to note is that to prevent the death of *E. coli* in the absence of substrate, 10 mL of EC culture was added to the main sample.

2.4. Laboratory Condition

The mean temperature of the laboratory during the test was 20-25°C which was measured by the digital thermometer daily (testo 104-IR).

2.5. Time of Exposure

In normal conditions, bacteria have the capability to move from side to side (to get food and nutrition). The mean swimming speed of *E. coli* in water is 1000 μm/s (23). As the distance between the center of two zones was 250 mm, the meantime to move from the center of zone LZ_1 to the center of zone LZ_2 (or in the right zone too) was approximately 2500 seconds or 42 minutes. In this way, the time for each series of experiments was 42 minutes, as calculated by a digital timer.

2.6. Methods of Experiments

After the MF exposure for 42 minutes in each run of the experiment, one loop of water in the reactor was taken from the center of each area and added to 35 mL (33.3 mL is necessary for examining MPN per 100 mL in 9 tubes) of distilled water using sterilized fildoplatin. After shaking the new sample, using a sterilized pipette, 0.1, 1, and 10 mL of contaminated water (water contain *E. coli*) were added to series (each series with 9 tubes) of sterilized tubes which contained EC culture media. These tubes were shaken well and incubated at 44°C for 48 hours. In the final experiment, the presence of turbidity and gas in Durham tubes were considered as a positive indicator and the number of *E. coli* in 100 mL were calculated by a standard table of counting microorganisms. MPN/100 mL of bacteria in each zone and control samples was determined to see the change in the number of *E. coli* in the case samples compared with each other and with the control sample. These experiments were done based on sections 9221D and 9221E of Standard Method for the Examination of Water and Wastewater (24).

This experiment was repeated for 9 series or runs of cases and control samples. The significant differences between the samples in terms of the mean number of colonies were evaluated using paired samples *t* test and ANOVA tests. The results are shown in Table 1. As there were not any statistically significant differences in MPN/100 mL of control samples at the beginning and the end of the experiment daily, the data collected in this section were omitted.

3. Results and Discussion

Based on the data showed in Table 1, the lowest mean number of *E. coli* was observed in zone Z (in the center of the reactor). On the other hand, the intensity of MFs in LZ_2 and RZ_2 was higher compared to the other zones and the variance of the number of *E. coli* was high as well.

The application of MTB in industrial processes and removing metals from the environment are considered as new courses of research and very few reports are published in these areas (25,26). The effect of MFs on biological systems differs based on the strength of MFs (from $10^7$to 10 T), type of bacteria, cell cultures, tissues, and animals (27).

The mean number of *E. coli* per 100 mL in all zones and the control sample is shown in Fig. 2.

The mean MPN/100 mL of *E. coli* in each area of the cylinder was compared simultaneously for investigating the effects of MFs on the attraction of *E. coli* to the area in which the intensity of MFs was high. Additionally, MPN/100 mL of *E. Coli* in each area of the reactor was separately compared with the control sample as follows.

3.1. Comparison of the Mean MPN/100 mL of *E. coli* in the Z Zone with All Zones and Control Sample

In Table 2, the mean MPN/100 mL of *E. coli* in the Z zone in the center of the reactor and MFs were determined and statistically compared with the right zone (RZ), the left zone (LZ), and the control samples.

As mentioned above, MTB are microorganisms which can align in and navigate along the MF lines (9). The reason for this property of MTB is that they biomineralize magnetite nanoparticles and organize them into chains that behave like a magnetic compass needle when put in the MFs (28,29).

3.2. Comparison of the Mean MPN/100 mL of *E. coli* in Control Sample and All Zones

The main goal of this study was to survey the effect of MFs on the movement of *E. coli* from an area with low intensity to an area with high intensity in case and control samples. The mean number of *E. coli* in all zones in comparison with the control sample is shown in Table 3.

The difference between the control sample and other zones (except for Z) was statistically significant ($P<0.000$). Therefore, it should be mentioned that the number of *E. coli* in the control sample during the examination did not change.

Magnetotactic cells passively swim and align along MF lines. Their accumulation at the edge of water drops in MFs were observed when employing a microscope (30,31).

3.3. Comparison of the Mean MPN/100 mL of *E. coli* in

Table 1. MPN/100mL of *E. coli* in all Zones

Parameter	Zone					
	LZ_2	LZ_1	Z	RZ_1	RZ_2	Control
N	9	9	9	9	9	9
Mean	21.33	5.30	6.97	8.87	23.78	10.98
Mean	20	3.6	6.1	3.6	27	11
Mode	15	3	3	3	20	15
Standard deviation	2.47	0.93	1.59	1.34	3.21	1.87
Variance	55	7.74	23.0	16.33	92.69	31.5
Minimum	14	3	3	3	5	3
Maximum	35	9.4	15	14	35	20
Sum	192	47.7	62.7	52.8	214	98.8

Table 2. Number of *E.coli* in the Z Zone Compared to All Zones and Control Sample

Zone	Std. Deviation	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
		Lower	Upper			
(Z –LZ_1)	6.02	-2.27	6.98	1.17	8	0.275
(Z – LZ_2)	10.63	-21.85	-5.51	-3.86	8	0.005
(Z – RZ_1)	4.83	-1.58	5.85	1.32	8	0.222
(Z – RZ_2)	7.78	-22.43	-10.48	-6.35	8	0.000
(Z – control)	12.29	-18.36	0.54	-2.17	8	0.61

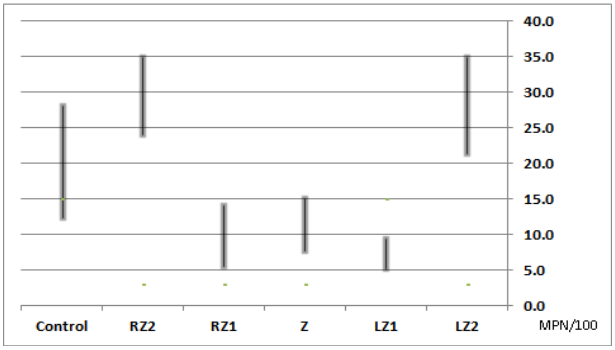

Fig. 2. MPN/100 mL of *E. coli* in All Zones and the Control Sample.

Symmetric and Non-symmetric Zones

In Table 4, the mean number of *E. coli* in symmetric (zones in which the distance between them and the center of the reactor or MFs are the same) and non-symmetric zones were compared.

The number of *E. coli* in symmetric zones (LZ_1-RZ_1) and non-symmetric (LZ_2-RZ_2) was not statistically the same. Additionally, the intensity of MFs did not differ. However, there were statistical differences between non-symmetric zones such as LZ_1 and LZ_2 and other non-symmetric zones, which are shown in Table 4. In a recent study about the swimming orientation of multicellular magnetotactic prokaryotes, no swimming of bacteria was reported using an MF intensity of 80 μT (32). However, this intensity might be low, as Haghi et al reported that moderate SMFs stimulated the growth of microorganisms (33). Removal of heavy metals, organic pollutants, and radionuclide from wastewater includes three types of the environmental applications of MTB for wastewater treatment (34). The removal of some pollutants such as heavy metals, radionuclides, oil, inorganic ion, organic contaminant and bacteria from the water body by magnetic separation is a commonly used technology for water treatment process (35,36).

Biotechnological and medical applications of MTB have been considered by some researchers. Drug delivery, imaging, antigen recovery, enzyme immobilization, detection or manufacture of magnetic cells, and pathogen detection are among these applications (37,38).

According to Loghin et al, the magnetic gradient can be used to detect the MTB and control their movements for drug transport application (39). One application of this study is the accumulation of bacteria in areas with a higher intensity of MFs, which may be due to the degradation of organic matter in wastewater.

Our study has two main limitations. First, the density of MFs in all zones was measured by formula, as there was not any detector (Tesla-meter or Gauss-meter) at hand during the study. Second, there were a limited number of studies about the effects of MFs on *E. coli* in the database, especially in the field of magnetotactic properties.

Table 3. Number of *E. coli* in Control Sample and All Zones

Zone	Std. Deviation	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
		Lower	Upper			
(Control – LZ_1)	6.85	1.85	12.39	3.17	8	0.014
(Control – LZ_2)	12.27	-0.54	18.6	2.17	8	0.049
(Control – RZ_1)	8.43	0.42	13.38	2.45	8	0.040
(Control – RZ_2)	9.66	-19.11	-4.26	-3.63	8	0.007

Table 4. Number of *E. coli* in all Zones and Control Sample

Zone	Std. Deviation	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
		Lower	Upper			
(LZ_1 _ LZ_2)	8.92	9.17	22.89	5.39	8	0.001
(LZ_1 – RZ_1)	4.94	-4.02	3.57	-0.13	8	0.896
(LZ_1 – RZ_2)	6.12	-23.58	-14.03	-9.08	8	0.000
(LZ_2 –RZ_1)	9.61	3.20	8.42	4.93	8	0.001
(LZ_2 – RZ_2)	12.87	-12.67	7.11	-0.65	8	0.536
(RZ_1 – RZ_2)	8.31	-24.98	-12.18	-6.70	8	0.000

4. Conclusion

Nowadays, the use of MTB in bioremediation, cell separation, biomineralization, and wastewater treatment is improving. Changes in the concentration of hormones, activity of enzymes, transcription of DNA, and transport of ions through membranes of cells are the main effect of MFs on biological systems. Although considerable progress in the study of MTB has been made, there are many questions that remain to be answered about the applications of MTB in the environment and industrial waste treatments. As *E. coli* is a known bacterium in environmental health engineering and other sciences, getting more information about this microbial indicator and its magnetotactic properties may help engineers to use this interesting microorganism for removing pollutants.

Bacteria respond to environmental signals. One of these responses is swimming towards the applied magnetic gradient (magnetotactic property). *E. coli* swims to the area in which the intensity of the MFs is higher. In our study, the MF intensity of 0.18 mT could spread 3.5 cm around the center of the cylinder. The MPN/100 mL of *E. coli* in the center of the reactor and control samples was measured, indicating significant differences compared to the zones which had a higher MF intensity. On the other hand, *E. coli* has the ability to swim from the area with a lower intensity of the MFs to the area with a higher intensity.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

Ethical Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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