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Growth of *Escherichia coli* Under Extremely Low-Frequency Electromagnetic Fields

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Abstract

The influence of extremely low-frequency (ELF) electromagnetic fields on *Escherichia coli* cultures in submerse fermentation was studied. The fermentation processes were carried out recycling the culture medium externally through a stainless steel tube inserted in a magnetic field generator (solenoid). The exposure time and electromagnetic induction were varied in a range of 1 to 12 h and 0.010 to 0.10 T, respectively, according to a Box-Wilson Central Composite Designs of face centered with five central points. Growth of *E. coli* could be altered (stimulated or inhibited) under magnetic field-induced effects. *E. coli* cultures exposed at 0.1 T during 6.5 h exhibited changes in its viability compared to unexposed cells, which was 100 times higher than the control. The magnetic field generator associated with the cellular suspension recycle is a new way of magnetic treatment in fermentation processes and could be appropriate to industrial scale up.

Index Entries: *Escherichia coli*; growth; cell culture; electromagnetic fields; biological effects.

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Introduction

The electromagnetic application in biotechnological processes is relatively recent and still very limited in industrial scale. For many years, scientists believed that electromagnetic fields of low frequency have not had any significant biological effects. In recent decades, several researchers demonstrated that the heat was not the only property potentially important in these radiations. Many scientific studies verified that electric and/or magnetic fields of extremely low frequency (<300 Hz) can influence the biological systems, could involve principal changes in the cellular proliferation *(1,2)*, stimulation of the ATP production *(3)*, changes in the flow of ions through the membranes *(4,5)*, and increases in the CO_2 formation in cellular cultures *(6)*.

Studies with a great variety of apparatus for generating electromagnetic waves indicated that the growth of bacteria and yeasts could be altered through this physical agent effect, independently of the employed equipment type *(7–11)*. In addition, electromagnetic fields have a positive effect on consumption of glucose and growth of *Escherichia coli (12)*. The cause of this result can be explained by an increase of glucose entering through the membrane as a result of the stimulated transport system and by shortening of the lag phase and excitement of the log phase. However, there is relatively little work in this area. The cell cultures used as experimental models have been mainly well characterized lineages, with varieties of genetic markers and ease of propagation.

Our research group has studied the genotoxic effect of magnetic field on *Aspergillus nidulans (13,14)*, on *Saccharomyces cerevisiae* growth *(9)*, and more recently in the nisin production *(15)* and the recombinant gp41 protein production using *E. coli* as a host. In the last case, we observed that the magnetic field altered the bacteria growth, which influenced the protein synthesis. We decided to study the biological effect of electromagnetic field on *E. coli* growth to verify the previous observation and provide a better understanding of the action of the electromagnetic field on the biological system. Also, *E. coli* was selected because of its wide use in molecular biological studies.

On the other hand, the fermentation processes were carried out by recycling the culture medium (cell suspension) externally through a stainless steel double U tube inserted in a magnetic field generator (solenoid), which is a single piece of equipment and would allow for an easy adaptation in a larger scale.

Materials and Methods

Microorganism

E. coli strain was obtained from the American Type Culture Collection (ATCC 25922) and maintained in nutrient agar (18 g/L), peptone (5 g/L), and meat extract (3 g/L) at 4°C.

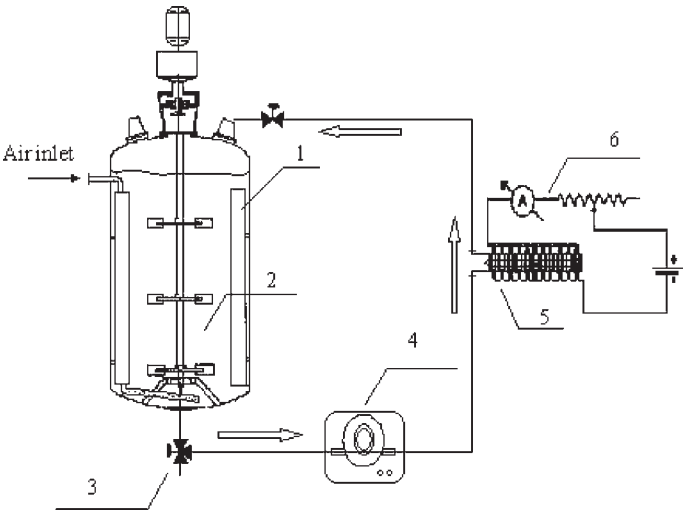

Fig. 1. Experimental setup for the magnetic field treatment of the cell suspensions of *Escherichia coli* C-600. (1) Fermenter, (2) cell suspension, (3) three-way valve, (4) peristaltic pump, (5) stainless steel double U tube and electromagnet, (6) current source. Drawing not to scale.

Inoculum Preparation

Inoculum was prepared in 500-mL Erlenmeyer flasks containing 300 mL of sterile medium. The composition consisted of yeast extract (5 g/L), tryptone (10 g/L), and NaCl (10 g/L). The Erlenmeyer flasks were kept in a rotary shaker at 200 rpm and 30°C for 24 h. The pH was adjusted to 7.0 with NaOH or HCl. All chemical reagents and bacteriological mediums used in this work were purchased from Merck and SIGMA, respectively.

Fermentation

Fermentation was started with 10% (v/v) inoculum of *E. coli* (approx 10^6 cells/mL) in a Marubishi stirred glass fermenter (Marubishi Co., Japan) at 25°C, 350 rpm, and 1.25 vvm. The working volume (1.2 L) contained 1.3 g of yeast extract, 6.5 g of tryptone, 13 g of casein, 50 g of glucose, 1.3 mL of $CaCl_2$ to 0.01 *M*, 1.3 mL of $MgSO_4$ to 0.1 *M*, and 130 mL of M910X (60 g/L of $NaHPO_4$, 30 g/L of K_2HPO_4, 10 g/L NH_4Cl and 5 g/L of NaCl). All media were sterilized at 121°C for 15 min and the pH was adjusted to 7.0 with NaOH or HCl 2 mol/L prior to sterilization.

The microbial culture in the fermenter was recycled through the magnetic field generator shown in Fig. 1, which allowed one to apply a magnetic field on the cell suspensions externally. This apparatus was described in a previous paper *(9)*, and consists of an electromagnet coupled to a stabilized current source of 2A to induce electromagnetic inductions (B) of 0.010 to 0.10 T. The cellular suspension was recycled through of a stainless steel double U tube of 0.005 m of internal diameter (ID) and 0.20 m of length,

disposed in the center of the electromagnet. The recycle loop was previously sterilized to avoid contamination. The velocity input on the fluid (cellular suspension) in the recycle was 1 m/s allowing it to work at extremely low frequency (ELF; range less than 300 Hz).

Statistical Analysis

The effects of the independent variables electromagnetic induction and exposure time were evaluated on the growth of *E. coli*, using a Box-Wilson Central Composite Design *(16)*, commonly called "Central Composite Design," of centered face for two variables. The experimental design matrix consists of 13 sets of coded conditions. It comprises a two-level full factorial design (2^2), four axial or star points, and five center points, all in duplicate. The independent variables were: x1-electromagnetic induction (0.010 to 0.10 T) and x2-exposure time (1 to 12 h). The responses were biomass, viability, and growth yield coefficients (Yx/s) expressed with respect to the substrate consumed. Analysis of variance (ANOVA) of the obtained data was performed through the Statistic software 5.0 (Stat Soft Co.). Significance levels of $p < 0.05$ were assumed to be statistically significant in this study.

Biomass Determination

The microbial biomass was quantified at the end of the fermentations. 10-mL medium samples were centrifuged at 2100*g*. The harvested cells were washed twice with distilled water and dried at 65°C and 8 kPa until they achieved constant weight in a vacuum oven.

Viability Determination

Viability was measured by the conventional technique of total viable bacterial counts determined by the colony forming units (UFCs). After exposure, a small amount (1 mL) of fermented medium sample was appropriately diluted and then plated on labeled Standard Methods Agar (SMA) plates, covered, and swirled to uniformly distribute the sample. After sitting undisturbed for approx 45 min, the Petri dishes were inverted and then incubated at 37°C. After 24 h of incubation, the colonies were counted. The *E. coli* numbers are represented as colony forming units (CFUs) m/L.

Results and Discussion

The fermentation of *E. coli* was evaluated for different operational conditions through a central composite designs (CCD), aiming to improve bacterial growth. The results obtained at the end of the fermentation process are illustrated in Table 1 and compared with the control experiment, which shows that the microbial biomass, the number of colonies, and the biomass/substrate yield (Yx/s) were influenced by the studied electromagnetic field conditions when compared to the control. High values were

Table 1
Results of the *E. coli* Growth Under Application of Electromagnetic Fields After 24 h of Fermentation, Using a Central Composite Design

Assay	X_1 (T)	X_2 (h)	Biomass (g/L)	Viability UFC × 10^{-8}	Yield (%)
1	0.010	1	5.95 ± 0.51	4.91 ± 0.37	14.48 ± 1.03
2	0.100	1	2.97 ± 0.43	8.10 ± 0.72	6.90 ± 0.78
3	0.010	12	11.02 ± 1.81	0.01 ± 0.00	27.28 ± 1.93
4	0.100	12	8.78 ± 0.72	9.26 ± 0.81	21.55 ± 1.86
5	0.010	6.5	4.06 ± 0.36	3.98 ± 0.23	9.70 ± 0.83
6	0.100	6.5	9.97 ± 0.89	10.76 ± 0.94	24.45 ± 0.98
7	0.055	1	3.50 ± 0.16	6.68 ± 0.52	8.18 ± 0.62
8	0.055	12	19.83 ± 1.61	0.01 ± 0.00	49.20 ± 2.53
9[a]	0.055	6.5	3.26 ± 0.14	7.93 ± 0.63	7.63 ± 0.63
10[a]	0.055	6.5	3.54 ± 0.23	8.01 ± 0.72	7.53 ± 0.23
11[a]	0.055	6.5	3.89 ± 0.33	7.56 ± 0.64	6.50 ± 0.36
12[a]	0.055	6.5	4.10 ± 0.28	7.83 ± 0.58	7.31 ± 0.52
13[a]	0.055	6.5	3.06 ± 0.16	8.21 ± 0.93	6.72 ± 0.64
Control experiment[b]			7.71 ± 0.43	0.01 ± 0.08	18.69 ± 0.72

Values are presented as mean ± standard deviation. [a]Central points used to estimate the experimental error. [b]Response corresponding to mean value of experiments performed in triplicate.

achieved for the microbial biomass and for the Yx/s at the largest exposure time (between 6.5 and 12 h). In the first case, 19.83 g of biomass per liter was obtained for the exposed culture to 550 Gauss for 12 h (Test 8) and the data indicate that increases in the exposition time resulted in improvement of the microbial biomass values. However, the viability of this test has no differences when compared to the control and there is not a direct relationship between viability and the biomass values.

When microbial cells are fixed in a solid culture medium several visible and isolated colonies are formed, but it is not possible to establish a direct relationship between the number of formed colonies and the number of cells. The correct relationship is made between the number of colonies and the colony forming units (UFCs), which can be as individual cells as characteristic groupings of certain microorganisms, where the dead cells that are part of the biomass are not quantified. In this context, the study of the electromagnetic fields effect relating to the viability seeks to verify the possible lethal effect of the field on *E. coli*. But, according to the results (Table 1) we cannot affirm that the electromagnetic field have no lethal effects on the bacteria cultures although in all the cases the viability (UFCs) was the same or superior to the ones obtained in the control test (unexposed cultures). Similar results have been reported *(17)*, but using *E. coli* under a static magnetic field at 0.030 T and researcher-observed alterations induced by the magnetic field in terms of increased cell prolif-

eration and changes in gene expression compared with the control. On the other hand, during exposure to the electromagnetic field, the temperature of the cell suspension was measured and no heating was observed in exposed culture mediums.

The Yx/s varied from 6.5 to 49.2%, with the best results achieved for the exposed culture to 550 Gauss for 12 h (test 8). However, inhibiting effects on the cellular growth were observed in the tests 1, 2, 5, 7, and 9–13, corresponding to the lowest Yx/s. In these cases, probably the consumption of the carbon source was not only reverted in microbial growth, suggesting that the exposition to the electromagnetic field can also have influenced the metabolic routes of this bacterium. In the other words, a certain amount of consumed substrate will not produce a proportional increase of biomass, because a portion of energy (ATP) originating from that consumption will be destined to the maintenance of the bacteria vital functions. However, in the other cases (tests 3, 4, 6, and 8), the exposure to electromagnetic field induced a variety of physiological and cellular responses that resulted in cellular proliferation. The exposure time was the most important variable that affected the Yx/s because its increases resulted in a significant increase in the response variable, and was statistically significant at a 95% confidence level.

Table 2 shows the effects of individual variables and their interactions. The statistical analysis indicates that, for the microbial biomass and for the Yx/s, only the exposure time effect was statistically significant, whereas in the case of the number of colonies, the two studied independent variables and their interactions were statistically significant. The quadratic regression coefficients for the response-surface models representing the microbial biomass, viability, and Yx/s are shown in Table 3. The coded second-order polynomial equation is given by Eq. 1, 2, and 3 according to the coefficients (Table 3) and they are the reduced models:

$$\text{Microbial Biomass} = 4.57 + 4.54X_2 + 4.11X_2^2 \quad (1)$$

$$\text{Viability (UFC)} = (7.53 + 3.2X_1 + 0.8X_1^2 - 1.74X_2 - 3.23X_2^2 + 1.52X_1X_2) \times 10^8 \quad (2)$$

$$Yx/s = 9.98 + 11.41X_2 + 11.28X_2^2 \quad (3)$$

A statistical test of the model was done by the Fisher's statistical test for analysis of variance (ANOVA) and the results are shown in Table 4. The ANOVA analysis of quadratic regression models obtained for the responses (biomass, viability [CFUs]), and Yx/s) demonstrates that they are not statistically significant, because in all the cases the $F_{calculated}$ values for the Lack of fit (Table 4) were much higher than F_{listed} values *(18)* and consequently the reduced models could not predict biomass, viability, and Yx/s under the studied experimental conditions range. However, the comparison of the *R-square* (*coefficient of determination)* value (0.9) to the viability model indicates a good agreement between the experimental and predicted values. Therefore, the *E. coli* growth under the electromagnetic field is a complex process and although the mechanism of action of these fields on

Table 2
Effects of Individual Variables and Their Interactions

	Biomass (g/L)		Viability UFC × 10^{-8}		Yx/s (%)	
Term	Effect	S.E	Effect	S.E	Effect	S.E
Mean	4.68*	0.17	7.53[a]	0.10	10.05[a]	0.21
X_1	0.23	0.33	6.41[a]	0.20	0.48	0.41
X_1^2	–0.79	0.48	1.59[a]	0.29	–00.47	0.60
X_2	9.07*	0.33	–3.74[a]	0.20	22.82[a]	0.41
X_2^2	8.51*	0.48	–6.46[a]	0.29	22.76[a]	0.60
$X_1 X_2$	0.37	0.40	3.03[a]	0.24	00.93	0.50

[a] Effect statistically significant ($p < 0.05$)
S.E., standard error.

Table 3
Regression Coefficients Obtained for All Dependent Variables ($p < 0.05$)

	Biomass (g/L)		Viability UFC × 10^{-8}		Yx/s (%)	
Term	Coefficient	S.E	Coefficient	S.E	Coefficient	S.E
Mean	4.57	0.15	7.53	0.10	9.98	0.19
X_1	–	–	3.20	0.10	–	–
X_1^2	–	–	0.80	0.14	–	–
X_2	4.54	0.16	–1.74	0.10	11.41	0.20
X_2^2	4.11	0.22	–3.23	0.14	11.28	0.28
$X_1 X_2$	–	–	1.52	0.12	–	–

S.E., standard error.

the biological system is not currently understood we believe that the magnetic field not only alters the membrane permeability, but also the culture medium as a result of the presence of ions. However, no pretreatment of the culture medium was carried out to verify the last hypothesis.

A representative response surface plot is shown in Fig. 2, showing the relative effect of exposure time and magnetic induction on viability, which for a high electromagnetic induction and approx 6.5 h exposure time, exhibited its maximum value. In addition, when cellular cultures are exposed for larger exposure periods, inhibition was observed. Similar results were observed in the yeast culture *(9)* under electromagnetic fields effect. On the other hand, other researchers *(19)* found that the viable number of *E. coli* was reduced to zero when the cells were exposed to pulsed magnetic field at 50 Hz and 0.15 T. Therefore, inactivation of *E. coli* has been reported *(20)* at 160 mT and 62 kHz during 16 h of exposure and, according to the authors, a considerable destruction level of *E. coli* cells was observed. Nevertheless, in this study no bactericidal effect was observed.

Table 4
Analysis of Variance for the Full Polynomial Models

Source of variation	Sum quadratic	Degrees of freedom	Mean quadratic	F test		R^2
				$F_{calculated}$	F_{listed}	
BIOMASS						
Regression (R)	177.88	2	88.94	8.22	4.10	0.62
Residual (r)	108.17	10	10.82			
Lack of fit (LF)	107.52	6	17.92	112.00	6.16	
Pure error (PE)	0.6464	4	0.16			
Total	286.05	12				
VIABILITY						
Regression (R)	118.22	5	23.64	12.46	3.97	0.90
Residual (r)	13.28	7	1.90			
Lack of fit (LF)	13.05	3	4.35	75.50	6.59	
Pure error (PE)	0.23	4	0.16			
Total	131.50	12				
BIOMASS/SUBSTRATE YIELD (Yx/s)						
Regression (R)	1193.01	2	596.50	8.41	4.10	0.63
Residual (r)	709.73	10	70.97			
Lack of fit (LF)	708.72	6	118.12	472.48	6.16	
Pure error (PE)	1.01	4	0.25			
Total	1902.73	12				

F_{listed} values are significant at 95% confidence level.

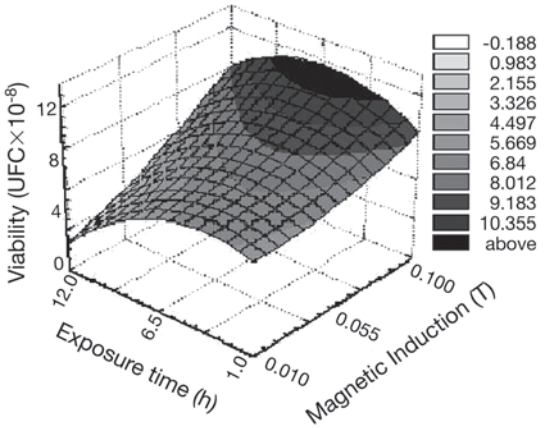

Fig. 2. Response surfaces for *E. coli* viability under ELF electromagnetic field with respect to the exposure time and magnetic induction.

Conclusions

The results showed that, in the studied range, the microbial biomass, the number of colonies, and the Yx/s of the fermentative process in the submerged phase of *E. coli* were influenced by the exposure to low frequency electromagnetic fields. The exposure time was the independent variable that presented the most important effects on the responses and these effects were specific for each one of the variables. The methodologies of factorial design and response-surface analysis were shown to be a useful tool for understanding how such parameters may affect the growth of *E. coli.*

In previous studies, we observed a 20% increase in the protein gp41 produced by *E. coli*. It is very difficult for this bacterium to secrete several recombinant products, because the Gram-negative bacteria has a double membrane structure and some of the products are accumulated in a periplasmatic space, but the cell growth is very important. Thus, a study about the magnetic field application on the *E. coli* for recombinant protein production is recommended.

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