MANIPULATION OF GROWTH CHARACTERISTICS OF YOGURT CULTURE BY APPLICATION OF AN ELECTROMAGNETIC FIELD

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by

David Michael Blicq

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BY

DAVID MICHAEL BLICQ

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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DEDICATION

FOR MOTHER

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GLOSSARY OF ABREVIATIONS AND SYMBOLS

Symbol	Unit	Definition
EMF	------	electromagnetic field
ELF	------	extremely low frequency
T	Tesla	magnetic flux density (1 Tesla = 10,000 Gauss)
G	Gauss	magnetic flux density
d.c.	Duty Cycle	percent EMF on/off
CFU	------	colony forming units

ABSTRACT

An electromagnetic field generating system was designed and developed which delivered a precisely defined EMF within two identical treatment coils. A variety of electromagnetic fields were applied to a number of microorganisms (Escherichia coli, Pseudomonas fluorescens, Bacillus stearothermophilus, Saccharomyces cereviseae, and Penicillium balaji) in order to determine a biologically effective range of EMF conditions. The effect of applied electromagnetic fields on the metabolism of a commercial yogurt culture was assessed by measuring pH, titrated acidity, plate counts of viable microorganisms, and high performance liquid chromatography analysis of organic acids produced during the initial 4 hr of fermentation. A significant increase in titrated acidity was observed in the treated culture exposed to an electromagnetic field of 60 Hz, 4.3 G, a 1% duty cycle and square waveform. Analysis of organic acids by high performance liquid chromatography attributed the elevation in titrated acidity to a 5.9% increase in the concentration of lactic acid; a primary fermentation product of Streptococcus thermophilis during the initial 4 hr of yogurt development. The enhancement of lactic acid production was determined to be frequency sensitive, over a frequency range of 50-70 Hz. No significant elevation

in the numbers of viable bacteria or the ratio of rods:cocci was observed through plate count methods, suggesting a change in culture metabolism rather than an elevation in the overall bacterial population was caused by the electromagnetic field. Supplementing the yogurt culture with calcium (5% that of indigenous levels) slightly decreased the metabolic enhancement of the lactic acid production, suggesting a possible role for calcium in the mechanism(s) of electromagnetic field effects on the yogurt culture.

2. INTRODUCTION

Recent research has established that electromagnetic fields (EMFs) can influence biological systems: at both the macro and microbiological levels (Postow, 1987). The effects of electromagnetic fields on biological systems vary greatly; ranging from direct inhibition of cellular growth to direct stimulation, in addition to numerous extremely subtle effects (Abelson, 1989; Andreev et al., 1987; Bauer, 1987; Chizhov et al., 1975; Crease, 1989; Gerencser et al., 1962; Greenebaum et al., 1982; Hamada et al., 1989; Moore, 1979; Morgan, 1989; Papatheofanis, 1987; Parkinson, 1985; Postow, 1987; Ross, 1990b). Electromagnetic field conditions which induce quite different effects in biological systems are often very similar in nature; typically differing in only a single characteristic of the applied electromagnetic field. The particular combination of electromagnetic field parameters (field strength, frequency, pulse duration, amplitude, field orientation) required to elicit an effect on a biological system can be referred to as an "electromagnetic field response window". The existence of electromagnetic field response windows has been well documented, although study of the characteristics which define electromagnetic field response windows is complicated by the fact that many biological responses to electromagnetic fields appear to be species or even strain-

specific (Achkasova et al., 1978; Lin-Xiang, 1990; Morgan, 1990; Postow, 1987). A specific electromagnetic field with a particular effect on a specific microorganism may exhibit an altered or even an opposite effect on a different microorganism (Moore, 1979).

Current research on potential applications of electromagnetic field technology has largely focused on the area of health, including the promotion of bone fusion (Iannacone et al., 1988) and ligament cell stimulation (Ross, 1990a). Investigations involving the effect of electromagnetic fields on microorganisms have for the most part been of an academic rather than applied nature, such as the study of bacteria in pure cultures (Moore, 1979; Morgan, 1990; Postow, 1987).

There were three objectives for this research. The initial objective was to design and assemble an apparatus capable of delivering a precisely defined electromagnetic field within a treatment coil. Secondly, a precisely defined electromagnetic field was to be applied in situ to an actively developing yogurt culture. The final objective was to assess the effectiveness of growth manipulation through the application of the electromagnetic field to the developing yogurt culture.

3. LITERATURE REVIEW

3.0 Electromagnetic Fields and Response Windows

The existence of biological effects of electromagnetic fields which occur only for specific combinations of electromagnetic field parameters has been well documented (Greenebaum et al., 1982; Greenebaum et al., 1979; Lin-Xiang, 1990; Moore, 1979; Postow, 1987; Ramon, 1981; Rathore and Goldsworthy, 1985a; Rathore and Goldsworthy, 1985b; Ross, 1990a; Saffer and Profenno, 1989; Seegal et al., 1989; Van Nostran, 1963; Verkin et al., 1976; Weaver, 1990; Zimmerman et al., 1990;). Biological effects of electromagnetic fields have been said to occur at "response windows": specific combinations of the parameters frequency, pulse duration, waveshape/waveform, and magnetic field intensity/amplitude. Electromagnetic field response windows for biological systems have been reported over a wide range of EMF conditions and have been detected even after exposure to extremely weak electromagnetic fields (Moore, 1979; Postow, 1987). Numerous biological effects of electromagnetic fields have been reported: ranging from direct stimulation or inhibition of biological activity to extremely subtle alterations in enzymatic, hormonal, and/or genetic systems (Neuman, 1987). Postow, (1987) reviewed numerous individual studies of biological effects of electromagnetic fields. It has been

determined that approximately 90% of investigations on biological effects of electromagnetic fields support the existence of either single or multiple frequency EMF response windows (Postow, 1987). Biological systems reported to possess EMF response windows include: cell lines of human, mouse, mouse neuroblastoma, rat, bacteria, chicken embryo, slime mold, guinea pig, guinea pig mitochondria, and monkey (Crease, 1989; Postow, 1987).

3.0.1 Response Windows and Microorganisms

Numerous researchers have reported the presence of electromagnetic field response windows in a variety of microorganisms, including both procaryote and eucaryote (Gerencser et al., 1962; Moore, 1979; Postow, 1987; Ramon, 1981). Selected research on electromagnetic field response windows in microorganisms is summarized in Table 1. In 1979, Moore reported that the growth of specific bacteria could either be stimulated or inhibited depending upon the nature of the particular electromagnetic field applied to the microorganisms. The electromagnetic field response windows reported were defined in terms of field strength, frequency, and waveshape of the applied pulsed magnetic field. Moore (1979) applied well-defined pulsed electromagnetic fields to a wide range of microorganisms in pure culture including organisms such as: Halobacterium halobium, Bacillus

TABLE 1. Reported electromagnetic field response windows for microorganisms: observed effects.

Author(s)	Organism(s)	EMF	Effect
Moore (1979)	H. halobium B. subtilis Ps. aeruginosa C. albicans S. typhimurium S. epidermis	0-0.3 Hz 50-900 G (various waveshapes)	stimulation, and/or inhibition
Ramon (1981)	Escherichia coli	60;600 Hz 0.003 T	40% inhibition (60 hr exp.)
Greenebaum et al. (1979)	P.polycephalum	45;60;75 Hz 0.01-0.2 mT	inhibition; (75 Hz)

subtilis, Staphylococcus epidermis, Pseudomonas aeruginosa, Candida albicans and Salmonella typhimurium. These microorganisms were grown in magnetic fields with frequencies ranging from 0.0-0.3 Hz, and with magnetic field strengths ranging from 50-900 G, while waveshapes of the applied electromagnetic fields were triangular, square, and sinusoidal. In general, Moore (1979) reported the response to electromagnetic field exposure to be greater for the Gram-negative bacteria than either the Gram-positive microorganisms or the yeast Candida albicans. He further reported maximum stimulation of microorganisms occurred after exposure to a relatively intense electromagnetic field with a magnetic field strength of 150 G. In contrast, an applied electromagnetic field of 300 G resulted in an inhibition of growth of exposed microorganisms. Moore (1979) also observed the frequency-dependent nature of the biological effects of the applied electromagnetic fields. Greatest stimulation of microbial growth was reported with an applied electromagnetic field of 0.3 Hz, while a static electromagnetic field (0 Hz) resulted in the maximum inhibition of bacterial development.

Ramon (1981) also examined the effects of electromagnetic fields on microorganisms. It was reported that dramatic reductions in Escherichia coli populations occurred after exposure to specific electromagnetic fields. Ramon (1981) described specific electromagnetic field

response windows using the parameters of signal frequency and magnetic field strength. E.coli cultures held at 0°C in phosphate buffer were exposed to applied electromagnetic fields with frequencies of 60 and 600 Hz and a measured field strength of 0.003 T. A decrease in E.coli populations of more than 40% that of control cultures was reported following 60 hr exposure to the electromagnetic field. It was concluded that further research would be required to adequately define the extent of the frequency response windows.

Populations of slime molds have been examined following exposure to specific electromagnetic fields (Greenebaum et al., 1979). The slime mold Physarum polycephalum was exposed to a variety of extremely low frequency (ELF) electromagnetic fields. Electromagnetic field exposures reported by Greenebaum et al. (1979) ranged from two months to five years duration and included signal frequencies of 45, 60 and 75 Hz, with magnetic field strengths ranging from 0.01-0.2 mT. It was reported that decreased respiration rate and a longer nuclear division cycle occurred in active cultures of P.polycephalum as a result of exposure to specific electromagnetic field. Maximum depression of cellular function occurred with an electromagnetic field characterized by a frequency of 75 Hz, with an applied field strength of 0.2 mT.

3.0.2 Response Windows in Complex Biological Systems

Electromagnetic response windows have been observed in relatively complex biological systems (Iannacone et al., 1988; Lin-Xiang, 1990; Ross, 1990a). Selected research on electromagnetic field response windows for complex biological systems is summarized in Table 2. Lin-Xiang, (1990) provided further evidence of electromagnetic field window effects while examining changes in c-myc and histone H2B transcripts of Human HL-60 cells following treatment with electromagnetic fields. Lin-Xiang (1990) exposed HL-60 cells to electromagnetic fields with frequencies ranging from 15-150 Hz, while field strengths were varied from 2-23 G. It was reported that histone H2B and c-myc levels increased significantly in cells exposed to the electromagnetic field as compared to control cultures. An electromagnetic field with a frequency of 45 Hz resulted in levels of histone and c-myc transcripts which were more than four times those found in unexposed controls. It was concluded that frequency is a major parameter in the definition of electromagnetic field response windows.

Ross (1990a) described electromagnetic field response windows while examining the proliferation of rabbit-ligament fibroblasts. Rabbit-ligament fibroblasts were exposed to extremely low frequency (ELF) electromagnetic fields which

TABLE 2. Reported electromagnetic field response windows in complex biological systems: observed effects.

Author(s)	Organism(s)	EMF	Effect
Lin-Xiang (1990)	Human HL-60 cells	15-150 Hz 2-23 G	increased histone H2B and c-myc 4X (45 Hz)
Ross (1990a)	Rabbit ligament fibroblasts	16,75,100 Hz variable field strength	stimulation and/or inhibition
Iannacone et al.(1988)	Rat costochondral junction (CCJ)	4.3 kHz 6.3-50 G	stimulation (34% 120 hr)
Yen-Patton (1988)	Endothelial cell monolayers	15 Hz 1.0 G	stimulation (20-40%)
Seegal et al. (1989)	Cerebrospinal fluid (CSF) pig-tailed macaques	60 Hz 0.9 G	inhibition (30%; 21 day)

resulted in a number of biological effects. Electromagnetic fields applied by Ross (1990a) were characterized by frequencies of 16, 75, and 100 Hz, and variable field strengths. It was reported that both stimulation and inhibition of the rabbit-ligament fibroblast occurred as a result of the electromagnetic field exposure, depending on the specific combination of the electromagnetic field parameters. Ross (1990a) concluded that the characterization of electromagnetic field response windows should include the parameters of signal amplitude, frequency, and DC magnetic field.

Iannacone et al. (1988) exposed the costochondral junction (CCJ) in 21-day-old rat to pulsed electromagnetic fields. Applied electromagnetic fields were varied to include a range of electric and magnetic field amplitudes. A pulsed electromagnetic field with a frequency of 4.3 kHz and a pulse width of 200 ms was utilized, while field strength was varied from 6.3-50 G. It was reported that stimulation of the in vitro growth plate of the costochondral junction occurred following application of the pulsed electromagnetic field, with a maximum growth increase of 34% after 120 hr of EMF exposure. Iannacone et al. (1988) characterized the electromagnetic field response windows using the parameters: frequency, pulse width (pulse duration), field strength, and signal waveshape/waveform.

Yen-Patton (1988) examined the effects of pulsed electromagnetic fields on the repopulation rate of denuded regions of endothelial cell monolayers. A significant increase in the growth rate of endothelial cell monolayers (20-40%) was observed following exposure to pulsed electromagnetic fields. The electromagnetic field applied by Yen-Patton (1988) consisted of a frequency of 15 Hz, a pulse duration of 0.2 ms, and a quasi-rectangular waveform with field strength measured at 1.0 G. Through the application of this particular electromagnetic field Yen-Patton (1988) was able to characterize a specific electromagnetic field response window which provided a 20-40% enhancement in the growth of endothelial cell monolayers.

Seegal et al. (1989) examined the neurochemical effects of 60 Hz electromagnetic fields applied to primates. It was reported that a significant decline in cerebrospinal fluid (CSF) in Macaca nemestrina (pig-tailed macaques) occurred following exposure to magnetic fields of 60 Hz, 0.9 G for three 21-day periods. The decrease in cerebrospinal fluid in exposed animals was 20-30%, and occurred at a specific frequency window of 60 Hz.

3.0.3 Response Windows and Genetic Apparatus

Response windows for electromagnetic fields have been observed in the genetic materials of a number of biological systems (Litovitz et al., 1990; Markov, 1987; Postow, 1987; Takahashi et al., 1986). Electromagnetic field effects on nuclear and/or genetic material are variable in nature, and include both inhibition and stimulation of RNA/DNA synthesis, as well as altered nuclear division (Goodman, 1989; Goodman, 1983; Postow, 1987). Selected research on electromagnetic field response windows in genetic apparatus is summarized in Table 3. Litovitz et al. (1990) described a model to explain transient augmentation of mRNA synthesis in mammalian cells following exposure to electromagnetic fields. It was reported that the change in reaction rate of mRNA transcription increases with the strength of the applied electromagnetic field. Litovitz et al. (1990) concluded that field strength was a significant determining factor in the definition of electromagnetic field response windows.

Takahashi et al. (1986) examined the effect of pulsing electromagnetic fields on DNA synthesis in mammalian cells in culture. Through the application of weak pulsing electromagnetic fields Takahashi et al. (1986) were able to significantly enhance DNA synthesis in chinese hamster V79 cells. Two specific electromagnetic field response windows were identified for the V79 hamster cells. An

TABLE 3. Reported electromagnetic field response windows and genetic materials: observed effects.

Author(s)	Subject(s)	EMF	Effect
Litovitz et al. (1990)	Mammalian mRNA	variable Hz variable G	enhanced mRNA synthesis
Takahashi et al.(1986)	Hamster V79 DNA	10;100 Hz 0.08 mT; 0.4 mT	inhibition DNA synthesis 80%
Greenebaum et al.(1979)	P.polycephalum	45;60;75 Hz	depressed nuclear division

electromagnetic field defined by a frequency of 10 and/or 100 Hz, a pulse width of 0.025 ms, and a magnetic field strength of 0.08 mT was reported to significantly enhance DNA synthesis. A similar electromagnetic field (10/100 Hz; 0.025 ms) differing only in magnetic field strength (0.4 mT) was found to depress DNA synthesis to 80% that of unexposed controls. This suggests a role for field strength in the definition of electromagnetic field response windows.

3.0.4 Role of Calcium on Electromagnetic Effects

Many of the investigations on biological effects of electromagnetic fields have reported changes in calcium metabolism in biological systems exposed to electromagnetic fields (Chen et al., 1988; Goodman, 1983; Lin-Xiang, 1990; Pilla, 1983; Postow, 1987; Ross, 1990a; Yen-Patton, 1988; Zimmerman et al., 1990). Changes in calcium levels and/or calcium channels during exposure of biological systems to electromagnetic fields are not entirely unexpected, "since the triggering event for calcium channel function is a voltage change" (Findlay and Evans, 1987). A summary of electromagnetic field effects involving alteration in calcium levels/pathways is described in Table 4.

Chen et al. (1988) reported the existence of calcium channels in the membranes of 3T3 fibroblasts, which included the presence of calcium channels in both treatment and

TABLE 4. Selected biological effects of electromagnetic fields exhibiting altered calcium metabolism.

Author(s)	Subject	EMF	Calcium Effect
Findlay and Evans (1987)	biological membranes	applied voltage	activation of Ca^{2+} channels
Chen et al. (1988)	3T3 fibroblasts	variable	activation of Ca^{2+} channels
Ross (1990a)	rabbit fibroblasts	variable	alteration of Ca^{2+} pathways
Zimmerman et al.(1990)	echinoderm zygotes	60 Hz; variable	influence on cytoplasmic Ca^{2+}
Pilla (1983)	various	variable	altered ionic absorption sites
Lin-Xiang (1990)	HL-60 cells	15-150 Hz 2-23 G	alteration of Ca^{2+} levels

control 3T3 cells. Chen et al. (1988) examined the voltage-sensitivity of the 3T3 fibroblast calcium channels. Voltage effects on the calcium channels in the 3T3 fibroblasts included significant alteration of normal cellular behavior. It was determined that activation of Ca^{2+} sensitive channels in fibroblasts may contribute to such Ca^{2+}-sensitive processes as control of secretion, shape change, motility, and phagocytosis. In addition to voltage-triggering of calcium channels, activation of calcium channels may also occur through the application of electromagnetic fields to biological systems (Ross, 1990a). Ross (1990a) reported changes in rabbit fibroblasts exposed to extremely low frequency electromagnetic fields. It was postulated that certain cellular communication pathways may be calcium dependent, such that biological effects of extremely low frequency electromagnetic fields may be the result of EMF-induced alteration in calcium pathways.

Zimmerman et al. (1990) reported changes in the development of sea urchins following exposure to a variety of magnetic fields, and suggested a role for calcium in the mechanism(s) of observed electromagnetic field effects. They hypothesized that a 60 Hz magnetic field might affect the first-division cycle in echinoderm zygotes through an influence on cytoplasmic calcium activity. Specific electromagnetic field interactions involving calcium have

been reported by other researchers (Lin-Xiang, 1990; Pilla, 1983).

Pilla (1983) suggested electromagnetic fields may interact with ionic adsorption sites to alter cellular behavior. Lin-Xiang (1990) also reported alterations in calcium levels in specimens exposed to electromagnetic fields, but stated it was unclear whether those changes were a direct result of electromagnetic field exposure.

3.1 Electromagnetic Field Description

The diverse nature of research on biological effects of electromagnetic fields has led to the development of a variety of experimental systems (Beiser, 1961; Goodman, 1989). It is therefore of paramount importance to describe electromagnetic fields using terminology which is both accurate and consistent. A summary of parameters which have been used to characterize electromagnetic fields is described in Table 5.

Goodman (1989) reported a number of parameters as crucial in characterizing electromagnetic field signals. These descriptive characteristics can be used to define either pulsed or single burst electromagnetic field signals, and include: waveform and/or waveshape, signal frequency (Hz), positive signal amplitude (mV), positive signal duration (ms), burst width (ms), negative space (between

TABLE 5. Reported descriptive parameters for electromagnetic field characterization.

Author(s)	Descriptive Parameters
Goodman (1989)	-waveform, waveshape -signal frequency (Hz) -positive signal amplitude (mV) -positive signal duration (ms) -burst width (ms) -negative spike (ms) -field strength (mT)
Ross (1990b)	-waveform, waveshape -pulse width (ms) -signal frequency (Hz) -signal rise/fall time (ms)
Pilla (1983)	-waveform, waveshape -signal frequency (Hz) -signal amplitude (G and/or T) -field orientation -culture vessel diameter (cm) -culture vessel height (cm)

peaks), negative spike (ms), and magnetic field strength (mT).

Ross (1990b) examined characteristics of pulsed electromagnetic fields as well as the principles used to describe these fields. These characteristics included: pulse width, repetition rate, waveshape/waveform (square, sinusoidal, ramped, triangular), and frequency composition. Ross (1990b) also stated that the rise/fall times of a signal must be taken into consideration, although signal rise/fall times are usually determined by the operational limitations of the equipment in each study. According to Pilla (1983), there are a number of parameters which characterize electromagnetic fields. These descriptive characteristics include: magnetic field uniformity (frequency, amplitude etc.), field geometry/orientation, culture vessel diameter, as well the height of the culture medium. Pilla (1983) also suggested that electromagnetic field parameters must not only be accurately described but must be kept constant during electromagnetic field experimentation in order to obtain meaningful experimental results. The constancy of electromagnetic field conditions was determined through assessment of magnetic field strength of EMFs applied both vertically and horizontally to petri plates containing various media. Pilla (1983) determined that parameters which must remain constant during system operation

include both vessel height/diameter, height of the culture medium, as well as cell concentration in cell-suspension studies.

3.2 Electromagnetic Field Generating Systems/Apparatus

Due to the wide scope of research on the interaction of electromagnetic fields with biological systems a number of EMF-generating apparatus have been developed. The majority of these systems permit control of specific electromagnetic field parameters, including electromagnetic field frequency, waveshape/waveform, pulse width/duration, and magnetic field strength. Summaries of apparatus used to establish and monitor electromagnetic fields are given in Appendices 1, 2 and 3.

Moore (1979) employed electromagnets of laminated silicone steel with 1500 turns of 25 gauge insulated copper wire per pole. Magnetic poles were kept 3.2 cm apart and were powered by a Kepco power supply (0-5. V, 0-1 A), while a Wavetek function generator produced the selected waveshape. Monitoring and maintenance of the electromagnetic signal was carried out using a Tektronic oscilloscope, with culture flasks placed directly between the poles of the electromagnets. Magnetic field strength was monitored directly with a Bell model 620 gauss meter (Moore, 1979).

Ross (1990a) employed an electromagnetic field generating system with electromagnets constructed of helmholtz coil pairs. Each helmholtz coil was fabricated with 70 turns of 18 gauge copper wire per pole. Magnetic fields were generated and controlled through a custom-designed amplifier which controlled both the frequency and amplitude of the electromagnetic signal, while culture flasks were placed directly between the coil pairs of the electromagnet. The electromagnetic field was monitored via a Bell model 620 gauss meter. Ross (1990a) also established an equation to calculate percent change in cellular proliferation for cells exposed to electromagnetic fields. According to Ross (1990b) the per cent change for fluctuating cellular populations in an electromagnetic field can be described as:

$$\text{per cent change} = (T-C)/C \times 100;$$

where T equals the number of cells in the population exposed to an electromagnetic field, while C represents the number of cells in the control. This calculation is particularly applicable for cellular populations which exhibit variable growth or initial concentrations which are not identical.

Goodman (1989) employed a commercially available Biosteogen System # 204 to generate a magnetic field between a pair of helmholtz coils (Electrobiology, NJ). Frequency of the electromagnetic field signal was controlled with a

Wavetek model 21 signal generator, while pulse rate was established using a Biosteogen system 100367 controller.

Lin-Xiang (1990) utilized a commercially available helmholtz coil (Electrobiology, NJ) to produce an electromagnetic field. A sinusoidal electromagnetic field was generated by a Wavetek signal generator, while power was supplied via a Radioshack amplifier.

In addition to the need for accuracy in monitoring electromagnetic field characteristics there is also a need to accurately monitor the input of thermal energy to biological systems during exposure to electromagnetic fields. Iannacone et al. (1988) stressed the importance of accurate temperature monitoring during application of electromagnetic fields to biological systems, stating sensitive and frequent temperature measurements must be made for all in vitro pulsed electromagnetic field experiments to prevent accompanying thermal stimulation artifacts.

3.3 Yogurt Culture

The term "yogurt" describes a variety of coagulated milk products. These products are the result of acid fermentation of milk by microorganisms such as Streptococcus thermophilus and Lactobacillus bulgaricus (Rasic, 1987; Singh and Shankar, 1984; Tamine and Robinson, 1985). The

fermentation is complex with S. thermophilus and L. bulgaricus interacting symbiotically to develop subtle sensory qualities in the final fermentation product (Sinha et al., 1987). The result of the milk fermentation is a highly acidic product with a final pH of 3.9-4.4, and which includes a range of total milk solids (Gupta and Prasa, 1989). Robinson (1990) states the composition of yogurt (percentage total solids) varies considerably, but in general, yogurt-type fermented milk products tend to contain between 12-15% total milk solids. Robinson (1990) describes the development of yogurt culture as a two-stage phenomenon. An initial production of lactic acid occurs as S. thermophilus enters the log phase of growth during the first 4 hr of fermentation. The remainder of the fermentation is dominated by L. bulgaricus which produces metabolites affecting the final flavor and aroma of the yogurt (Kosikowski, 1982). Robinson (1990) further identifies three important criteria which largely determine the final composition of the fermented milk product: the specific starter microorganism(s), the precise conditions employed during the fermentation, as well as the composition and treatment of the basic mix. Specific microbial activity during the yogurt fermentation was singled out as the factor having greatest influence on product development (Khan, 1990; Robinson, 1990; Tamine and Robinson, 1985). Commercial

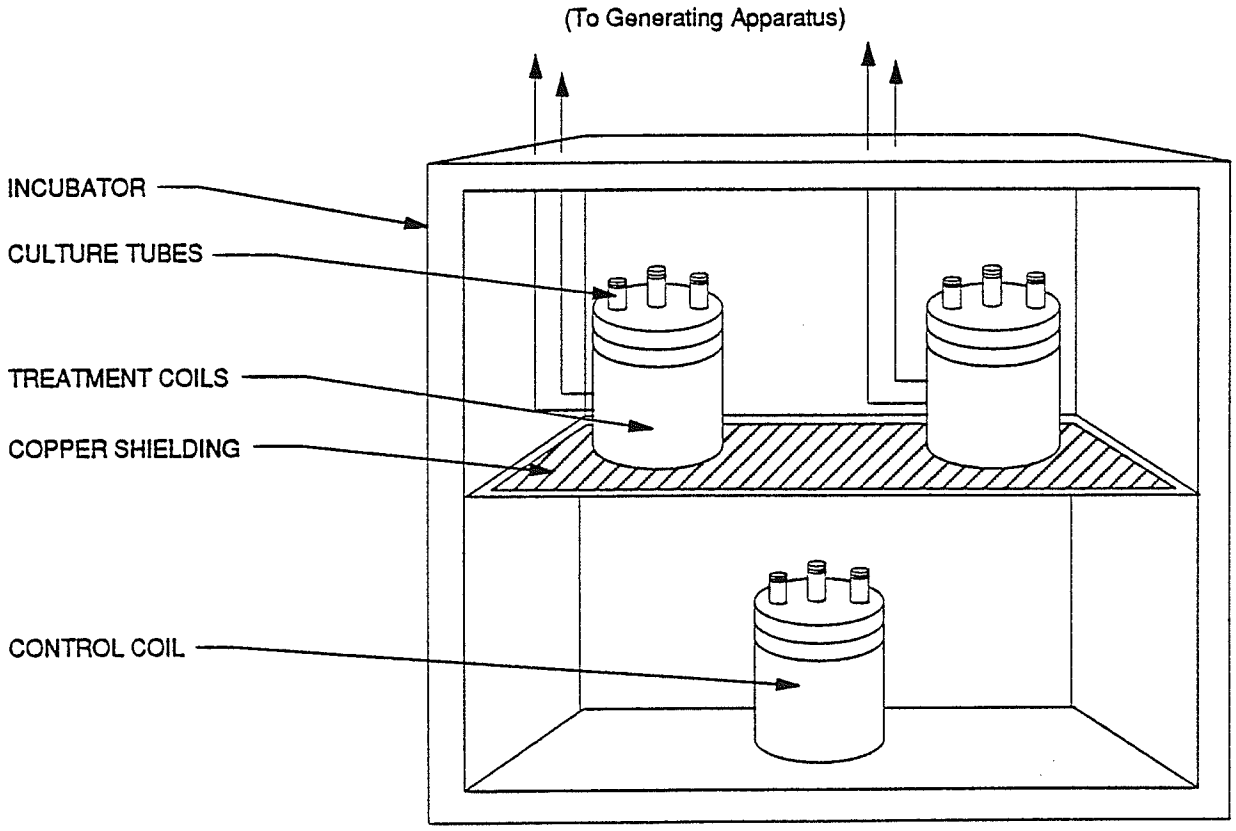
yogurt starter culture CH2 (Dri-Vac Lactic Culture, Chr. Hansen's Laboratory Inc.) contains selected strains of S.thermophilus and L.bulgaricus.

4. MATERIALS AND METHODS

4.0 Yogurt culture and Maintenance

A commercial Yogurt starter culture CH2 (Dri-Vac Lactic Culture, Chr. Hansen's Laboratory Inc.) containing selected strains of S. thermophilus and L. bulgaricus was obtained through the University of Manitoba Dairy. CH2 lactic culture consists of a 1:1 ratio of S. thermophilus and L. bulgaricus. A preparation of 12% skim milk was autoclaved at 88°C for 50 min to eliminate bacterial contamination. An inoculum of 2% (volume/volume) from a 24 hr yogurt culture was used to inoculate autoclaved 12% solids skim milk. The inoculated skim milk was agitated to ensure equal distribution of the starting inoculum. Sterilized test tubes were each filled with 10mL of the inoculated skim milk preparation. Four culture tubes were placed in racks within each of the electromagnetic field generating coils (Figure 1). Calcium-enriched yogurt was prepared by adding calcium chloride (Fisher Scientific) to 12% solids skim milk for a final calcium increase of 5% over the naturally-occurring level of 1293 mg/100 g (USDA, 1963).

FIGURE 1. Schematic diagram of culture placement within control and electromagnetic field generating coils.



4.1. Growth Assessment

4.1.1. pH/Titrated Acidity Determination

The pH of the yogurt culture was measured using an Acumet 925 pH/ion meter according to the standard AOAC method (AOAC, 1984; Williard and Merrit, 1968). Yogurt samples were removed from the treatment and control electromagnetic field coils and measured at timed intervals. Measurement of titratable acidity was carried out using 0.1N NaOH with several drops of phenolphthalein indicator following the AOAC (1984) standard method. Titratable acidity was expressed as percent lactic acid (Singh and Shankar, 1984).

4.1.2. Enumeration of Microorganisms

Total viable microorganisms (CFU/mL) were determined using nutrient agar following standard plate count methodology. Plates were incubated at 42°C and enumerated after 48 and 72 hr. Enumeration of colonies was facilitated through illumination of each petri plate on a Quebec colony counter. Distribution of the S. thermophilus to L. bulgaricus was determined using modified commercially available MRS agar (Difco) following standard plate count methods. The MRS medium was modified by adding 0.1% Tween 80 and 50 µg/mL of 2,3,5-triphenyltetrazolium (Sanchez-Banuelos et al., 1982; Matalon and Sundine, 1986). Plates were enumerated after 24 and 48 hr at 37°C using a Quebec colony counter.

4.1.3. HPLC Analysis of Organic Acids

Analysis of organic acids in yogurt samples was carried out using a modified method of Marsili et al. (1981). Organic acid extraction was carried out by adding 5 g yogurt to 25 mL solvent (Khan, 1990). The solvent consisted of a 4:1 mixture of acetonitrile:deionized water. The yogurt/solvent mixture was agitated by vortex blending for 30 s. Samples were then centrifuged at 7000 x g for 10 min. The supernatant was filtered through a Millipore HV 0.45um preparatory filter. The HPLC system consisted of a Waters model 510 pumping system, a Shimadzu SPD-6A UV spectrophotometric detector, a Hewlett Packard HP3396 Series II integrator and an HP Peak-96 information manager computer data acquisition system. Analysis was carried out using a Biorad HPX-87H organic acid column. The mobile phase was 0.006M H_2SO_4, and a flow rate of 0.6 mL/min was used. The column operating temperature was 69°C as determined by Khan (1990). Standard organic acids included: lactic, citric, pyruvic, orotic, propionic and acetic acid. The integrator was calibrated to identify organic acids based on retention time, while quantification of each organic acid was done using peak height.

4.1.4. Temperature Monitoring and Control

Temperature inside treatment and control tubes was

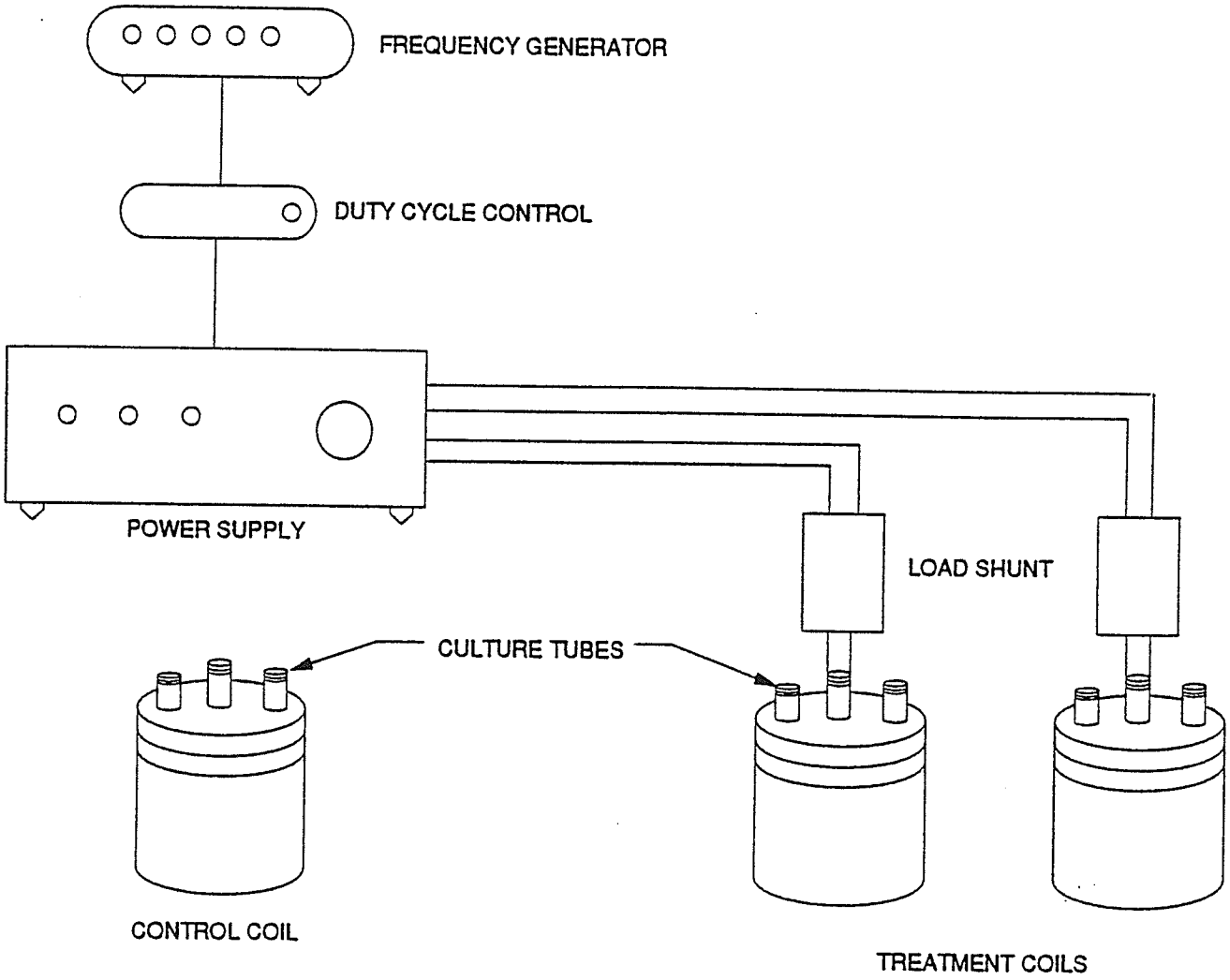
monitored and recorded using thermocouples wired directly into a computer data acquisition system from Omega Engineering (Alpha Products). Temperature measurements were confirmed with a Tegam model 871A portable thermocouple system. Thermocouple probes were placed inside the tubes of yogurt positioned within both the treatment and control EMF coils. To ensure equilibrium temperatures were maintained throughout the incubator a circulatory fan powered by a Powerstat variac was used to promote even air/temperature distribution. The effect of additional thermal energy on the growth of the microbial culture was determined by comparison of plate counts carried out on identical samples of yogurt culture grown at 42°C and 43°C for 4 hr. A schematic diagram of the computer temperature monitoring apparatus and placement of thermocouples is given in Appendix 4.

4.2. Electromagnetic Field Generating Apparatus

4.2.1. Power Supply

Power was supplied by a pair of Cyrus One integrated amplifiers. Each amplifier possessed a maximum power consumption of 100 W. One amplifier was utilized as a power supply for each pair of treatment coils. A schematic diagram of the assembled electromagnetic field generating apparatus is shown in Figure 2.

FIGURE 2. Schematic assembly of electromagnetic field generating apparatus showing frequency generator, duty cycle controller, power supply, load shunts and coils.



4.2.2. Frequency Control

Frequency was controlled with a Dynascan 3010 function generator (Precision Instruments). In the second electromagnetic field generating apparatus frequency was controlled with a frequency-limiter fabricated within the laboratory. The frequency limiter permitted frequency adjustment between 30 - 100 Hz.

4.2.3. Waveshape Control

Waveshape was controlled with a Dynascan 3010 function generator. Waveshape could be established as sinusoidal, rectangular, or linear and ramped in nature.

4.2.4. Pulse Duration

Pulse duration was controlled by two pulse-limiting devices manufactured in the laboratory. Pulse duration was established with duty (on/off) cycles of 1, 10, and 50%.

4.2.5. Electromagnetic Field Coils

Electromagnetic field generating coils were fabricated by winding insulated 32 gauge copper wire around PVC pipe in a linear fashion. The PVC pipe had a vertical height of 10.5 cm, a 10 cm interior diameter, and a wall thickness of 0.75 cm. Connections with the electromagnetic field generating apparatus were made using Realistic (Tandy) adapters which

were connected to the positive and negative leads of each coil. An alternative variety of EMF generating coil was also fabricated. The second variety of coil had a height of 3.0 cm and utilized 50 windings of 32 gauge insulated copper wire.

4.2.6. Load Control

In order to provide the minimum electrical load necessary to operate each amplifier, each coil was fitted with a resistance shunt. The resistance shunts were positioned directly between the integrated power amplifier and each coil. The shunts imparted a resistance of 2.4 ohm to each line and were identical for all treatment coils.

4.2.7. Sample Shielding

A 2 mm thick two-sided copper plate (30 cm x 40 cm) was used to isolate the electromagnetic field treatment area from the control cultures. The copper shielding was placed horizontally between the EMF treatment and control coils. Measurement of magnetic field strength in the control culture area was carried out to ensure that only the natural background of magnetic flux was present.

4.3. Electromagnetic Field Measurement

4.3.1. Field Strength

The strength of the magnetic field was measured using a Bell model 4048 Gauss/Tesla meter equipped with a transaxial probe. The electromagnetic field probe was calibrated using a Bell calibration tube. The transaxial probe was placed at 90° to the horizontal in order to measure the electromagnetic field generated within each coil. To measure the field strength accurately the EMF was established as a flat signal with the duty cycle set at 100%. Field strength was measured at the center and the sides of each coil. The highest measurable magnetic field was determined to be the electromagnetic field strength within each coil.

4.3.2. Waveshape

Electromagnetic field waveshape was determined by the Dynascan 3010 function generator. Waveshape was confirmed through visual observation using an LA-545 oscilloscope.

4.4. Preliminary EMF Response Window Investigations

During the development of the electromagnetic field generating apparatus a series of preliminary experiments were carried out. These early investigations were intended to calibrate and assess equipment effectiveness in generating and delivering specific electromagnetic fields, as well as determine a biologically-active range of electromagnetic field conditions from results reported from other investigations. Electromagnetic fields were generated by the apparatus described in section 4.3. Organisms exposed to electromagnetic fields included Pseudomonas fluorescens, Bacillus stearothermophilus, Escherichia coli, Saccharomyces cereviseae, and the fungus Penicillium balaji. Microorganisms were exposed to precisely defined electromagnetic fields in liquid culture and/or on agar plates.

Cultures of Pseudomonas fluorescens, Bacillus stearothermophilus, Escherichia coli MP180, and Saccharomyces cereviseae were obtained through the culture collection of the Microbiology Department, University of Manitoba. Cultures of Penicillium balaji were obtained from Philom Bios laboratories (Saskatoon, Saskatchewan). All cultures were maintained on plates and slants of tryptic soy agar (Difco) and standard methods agar (Difco) incubated at 37°C, with the exception of P.balaji (maintained on plates and slants of czapek dox agar (Difco), and S.cereviseae

(maintained on plates and slants of wort agar and yeast/mold broth (Difco)). The effect of electromagnetic fields of 30 and 60 Hz, 4.3 G, 1.0% duty cycle and square waveform was assessed by visual comparison of colony development using a Quebec colony counter. Spread plates of Ps. fluorescens (approximately 10^2 CFU/mL) were exposed to the electromagnetic fields for 10 days. EMF exposures were conducted at 7°C, in order to delay culture development and maximize differences between control and EMF-exposed cultures. Spread plates of B. stearothermophilus were also exposed to EMFs of 30 and 60 Hz, with exposures conducted at 25°C in order to delay culture development. All plates were examined daily.

A second series of experiments were carried out in order to assess the effect of an electromagnetic field on the development of liquid cultures of microorganisms. During these experiments cultures of Ps. fluorescens, E. coli, P. balaji, and S. cereviseae were grown in liquid cultures in 250 mL Klett flasks on an Eberbach rotary shaker using low speed rotation. Ps. fluorescens, E. coli, and P. balaji were each inoculated (10^1 CFU/mL) in nutrient broth (Difco) and incubated at 25°C while exposed to EMFs of 60 Hz, 4.3 G, 1.0% duty cycle and square waveform. Cultures of E coli were also exposed to a similar EMF with a frequency component of 600 Hz. Culture flasks were placed within EMF-generating

coils on the rotary shaker and an electromagnetic field was applied to the developing cultures. Control cultures were placed within non-active EMF-generating coils. Electromagnetic field exposures were carried out for 24 hr at 25°C, with the exception of P. balaji which was incubated for 7 days at 25°C. Culture development was monitored through turbidity measurements of control and EMF-treated cultures using a Klett-Summerson Turbidimeter/Colorimeter utilizing a green filter with wavelength at 500-570 nm. Cultures were removed from the EMF-generating apparatus at timed intervals in order to monitor culture development. The development of P.balaji was determined through measurement of biomass (dry weight) following 7 days incubation.

During the final preliminary study dilutions of S. cereviseae (10^4 CFU/mL) were inoculated into 100 mL flasks containing 0.1% peptone. Plate counts were conducted for control and treated samples incubated at 25°C for 7 days and exposed to an electromagnetic field of 60 Hz, 4.3 G, 1.0% duty cycle, and square waveform.

Temperature was monitored using a Tegam 871A portable thermocouple. Temperature measurements were recorded with a computer data acquisition system (Omega Engineering/Alpha Products).

5. RESULTS

5.0. Measured pH / Titrated Acidity

A decrease in the pH of the yogurt culture was observed during the 4 hr incubation period. A difference in pH values was also observed between the treated and control cultures. Figure 3 shows the mean value of three replicates for each coil. The difference in pH values between the control and treated cultures was not significant using a Duncan's multiple range test ($p<0.05$, from Appendix 5). Titratable acidity increased with incubation time for both the control and treated cultures (Figure 4). A difference in titratable acidity was observed between the control and treatment tubes. The difference in titratable acidity between the control and treatment tubes was significant using a Duncan's multiple range test ($p<0.05$, from Appendix 6) probably because of the greater sensitivity of the titration method. Mean titratable acidity of treated cultures was 8.9% more than controls.

Figure 3. pH of yogurt samples as a function of incubation time at 42°C. Electromagnetic field: 60 Hz, 4.3 G, 1.0% d.c. (EMF(1); EMF(2) represent duplicate treated samples).

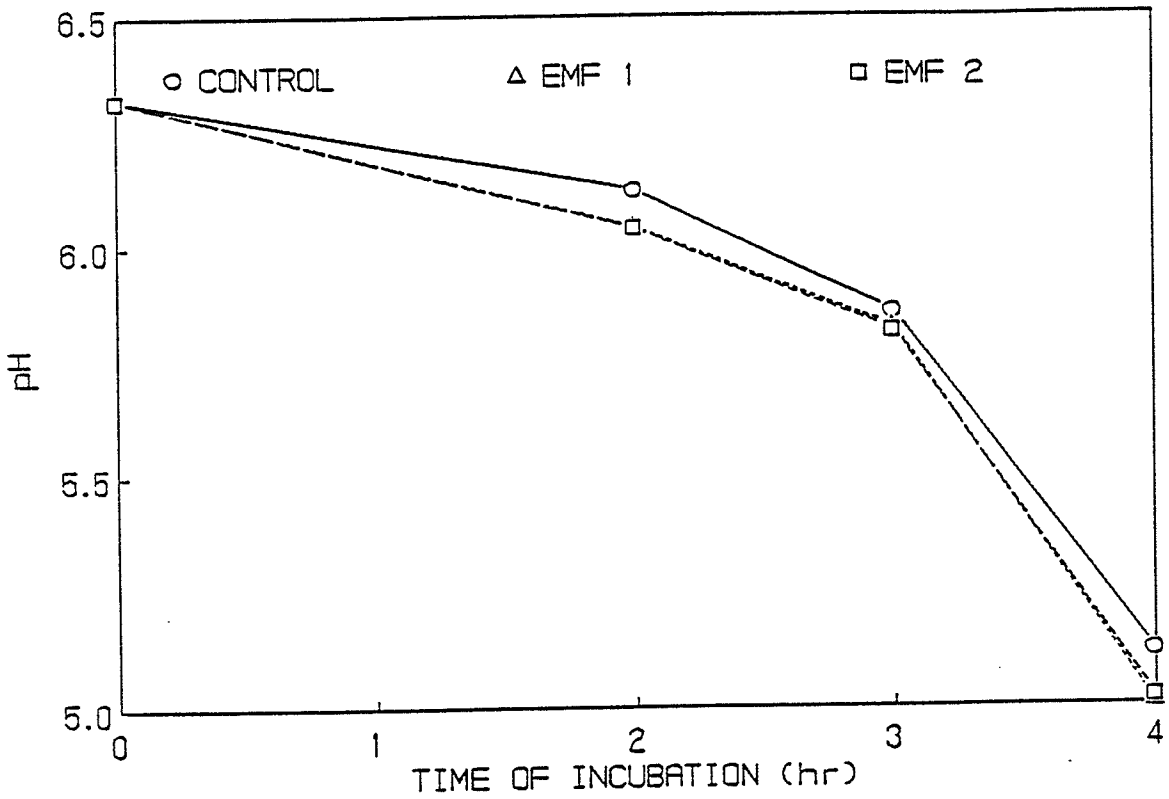
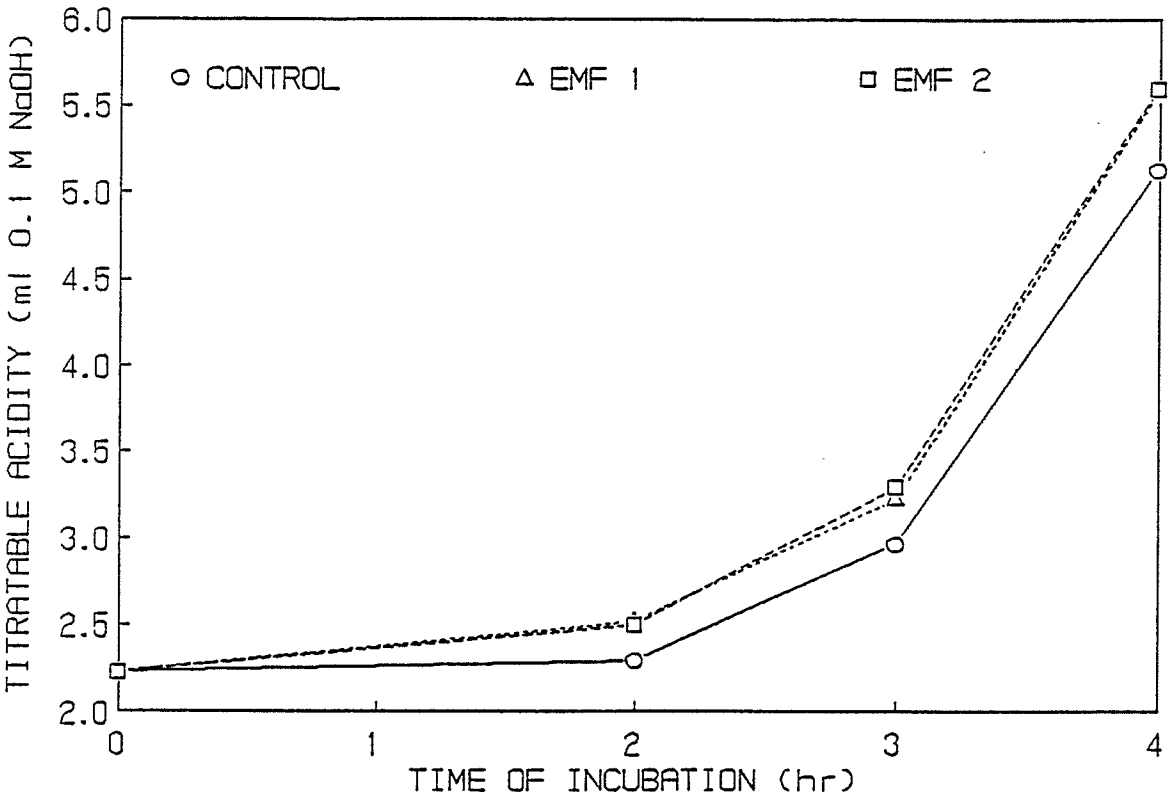


Figure 4. Titrated acidity of yogurt samples as a function of incubation time (hr) at 42°C. Electromagnetic field: 60 Hz, 4.3 G, 1% d.c. EMF(1), EMF(2) represent duplicate treated samples.



5.1. HPLC Analysis of Organic Acids

Analysis of organic acids by HPLC revealed increased concentrations of lactic acid in the EMF-exposed yogurt culture after 4 hr incubation. Lactic acid concentrations for yogurt culture exposed to electromagnetic fields of varying frequency are given in Tables 6-11. Mean lactic acid concentration as a function of frequency of the applied electromagnetic field is shown in Figure 5. The per cent change in lactic acid concentration as a function of frequency of the applied electromagnetic field is shown in Figure 6. No change in concentration was observed for the remaining organic acids (acetic, pyruvic, propionic, citric, orotic) following 4 hr exposure to electromagnetic fields of 50, 60 or 70 Hz, suggesting homo-fermentative conditions existed during the initial 4 hr of fermentation. Specific concentrations of the organic acids in the yogurt culture varied with the frequencies of the applied electromagnetic field. A 1.3% decrease in the level of lactic acid was observed for the treated culture following 4 hr exposure to the 50 Hz EMF, while application of the 60 Hz EMF resulted in an increase in lactic acid of 5.9% after 4 hr. Lactic acid levels in the treated culture following 4 hr of exposure to a 70 Hz electromagnetic field increased by 0.2%. Application of a 60 Hz electromagnetic field to a calcium-fortified yogurt culture (+5% calcium) resulted in an increase in lactic acid

TABLE 6. HPLC determination of lactic acid levels (mg/mL)* in control and treated samples following 4 hr incubation at 42°C, and exposure to EMF of: 50 Hz, 1% d.c., 4.3 G.

		Extraction Series				
Frequency	Sample	(A)	(B)	(C)	(D)	(E)
50 Hz	Initial	0.228	0.303	0.245	0.259	0.179
	Control	1.211	1.192	0.976	1.764	1.562
	EMF(1)	1.167	1.115	0.990	1.782	1.600
	EMF(2)	1.217	1.200	0.981	1.767	1.412
	EMF(av)	1.192	1.158	0.986	1.773	1.508
(T-C)/C x100		-1.6%	-2.9%	-1.0%	+0.5%	-3.6%
Total Mean % Change:		-1.3%				

* (based on duplicate HPLC runs for each extracted sample)

TABLE 7. HPLC determination of lactic acid levels (mg/mL)* in control and treated samples following 4 hr incubation at 42°C, and exposure to EMF of: 60 Hz, 1% d.c., 4.3 G.

		Extraction Series				
Frequency	Sample	(A)	(B)	(C)	(D)	(E)
60 Hz	Initial	0.245	0.209	0.220	0.272	0.304
	Control	1.563	1.456	1.256	1.489	1.164
	EMF(1)	1.648	1.583	1.430	1.556	1.249
	EMF(2)	1.669	1.532	1.243	1.543	1.206
	EMF(av)	1.660	1.558	1.337	1.555	1.230
	(T-C)/C x100	+6.1%	+7.0%	+6.4%	+4.3%	+5.5%
	Total Mean % Change: +5.9%					

* (based on duplicate HPLC runs for each extracted sample)

TABLE 8. HPLC determination of lactic acid levels (mg/mL)* in control and treated samples following 4 hr incubation at 42°C, and exposure to EMF of: 70 Hz, 1% d.c., 4.3 G.

		Extraction Series				
Frequency	Sample	(A)	(B)	(C)	(D)	(E)
70 Hz	Initial	0.258	0.221	0.242	0.264	0.277
	Control	1.106	1.182	2.009	1.372	1.124
	EMF(1)	0.992	1.075	1.456	1.413	1.204
	EMF(2)	1.113	1.264	2.623	1.278	1.050
	EMF(av)	1.130	1.170	2.040	1.345	1.120
	(T-C)/C x100	+2.2%	-1.1%	+1.5%	-1.9%	+0.3%
	Total Mean % Change: +0.2%					

* (based on duplicate HPLC runs for each extracted sample)

TABLE 9. HPLC determination of lactic acid levels (mg/mL)* in control and treated samples of calcium-enriched yogurt following 4 hr incubation at 42°C, and exposure to EMF of: 60 Hz, 1% d.c., 4.3 G.

Frequency	Sample	Extraction Series (A)	(B)	(C)	(D)	(E)
60 Hz (5% Ca^{++})	Initial	0.222	0.260	0.292	0.217	0.257
	Control	1.045	1.085	1.093	1.421	1.469
	EMF(1)	1.096	1.125	1.145	1.532	1.577
	EMF(2)	1.081	1.139	1.120	1.440	1.557
	EMF(av)	1.089	1.132	1.133	1.486	1.567
	(T-C)/C x100	+4.2%	+4.3%	+3.6%	+4.6%	+6.6%
	Total Mean % Change: +4.7%					

* (based on duplicate HPLC runs for each extracted sample)

TABLE 10. Mean concentration of lactic acid (mg/mL) as a function of frequency of applied EMF following 4 hr incubation.

	Frequency(Hz)			
Sample	50	60	70	60(5% Ca^{++})
Initial	0.243	0.250	0.252	0.250
Control	1.341	1.386	1.359	1.223
EMF(1)	1.331	1.493	1.228	1.295
EMF(2)	1.315	1.438	1.466	1.267
EMF(av)	1.320	1.468	1.346	1.281

(mg/mL based on mean of five determinations)

Figure 5. Lactic acid concentration (mg/mL) in yogurt samples as determined by HPLC analysis as a function of frequency of electromagnetic field treatment. EMF: 4.3 G, 1.0% d.c.

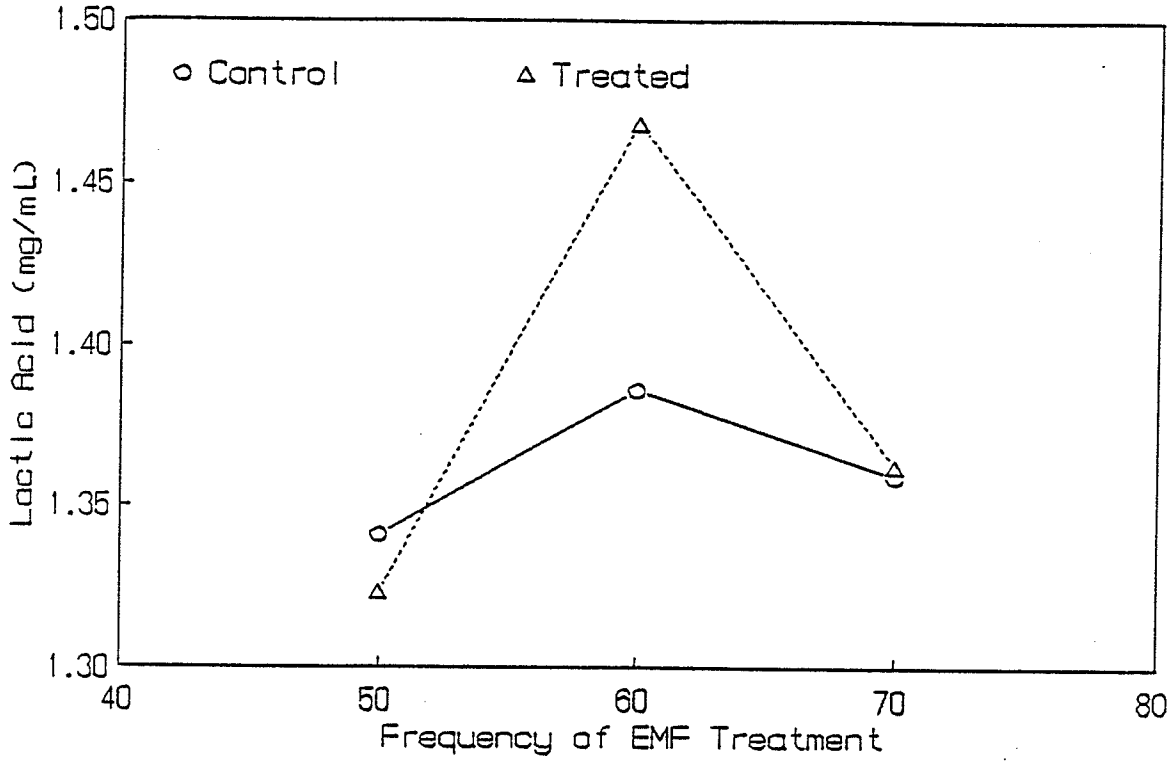
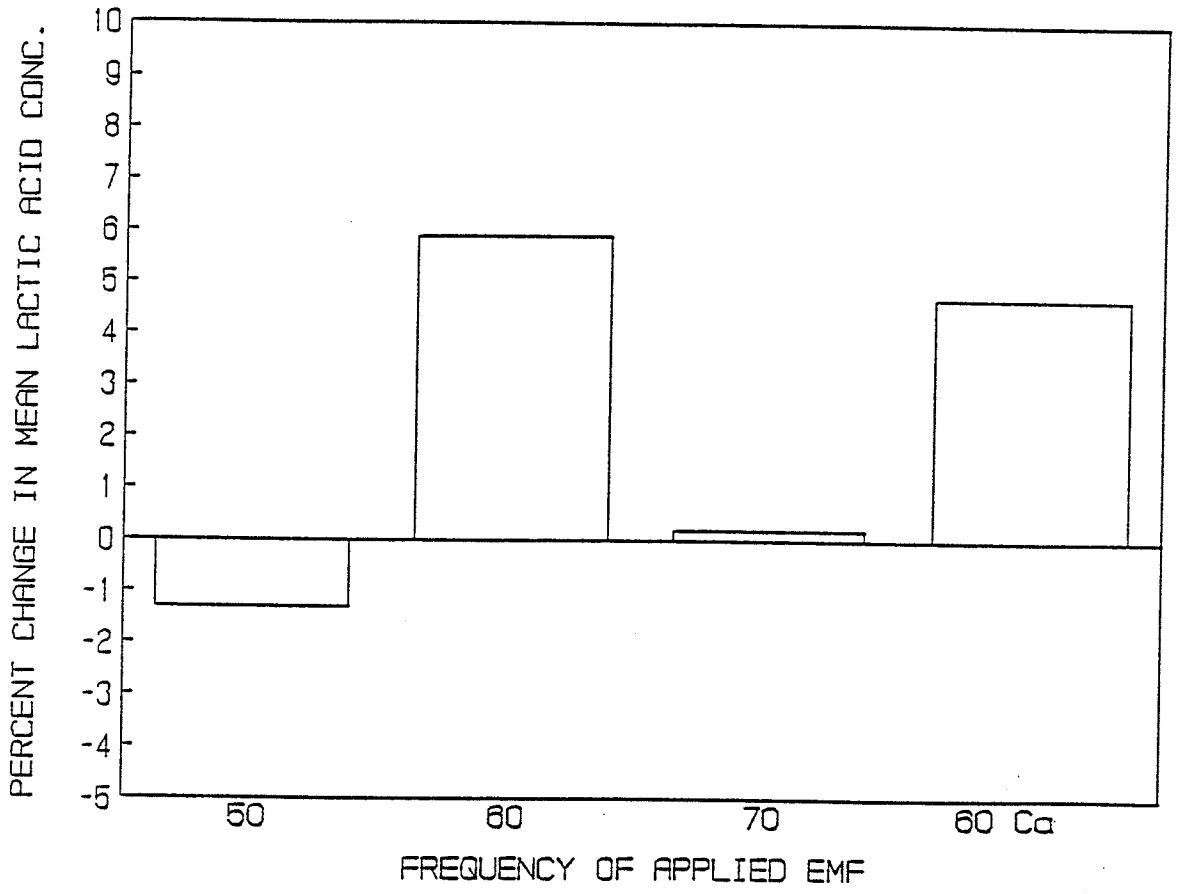


TABLE 11. Per cent change in mean lactic acid concentration (mg/mL)* as a function of frequency of applied EMF following 4 hr incubation.

Frequency(Hz)	Control	Treated	% Change
50	1.341	1.323	- 1.3%
60	1.386	1.468	+ 5.9%
70	1.359	1.346	+ 0.2%
60 (5% Ca^{++})	1.223	1.281	+ 4.7%

* (Based on 5 samples per each applied EMF frequency)

FIGURE 6. Summary of percent change in lactic acid concentration as a function of frequency of applied EMF.



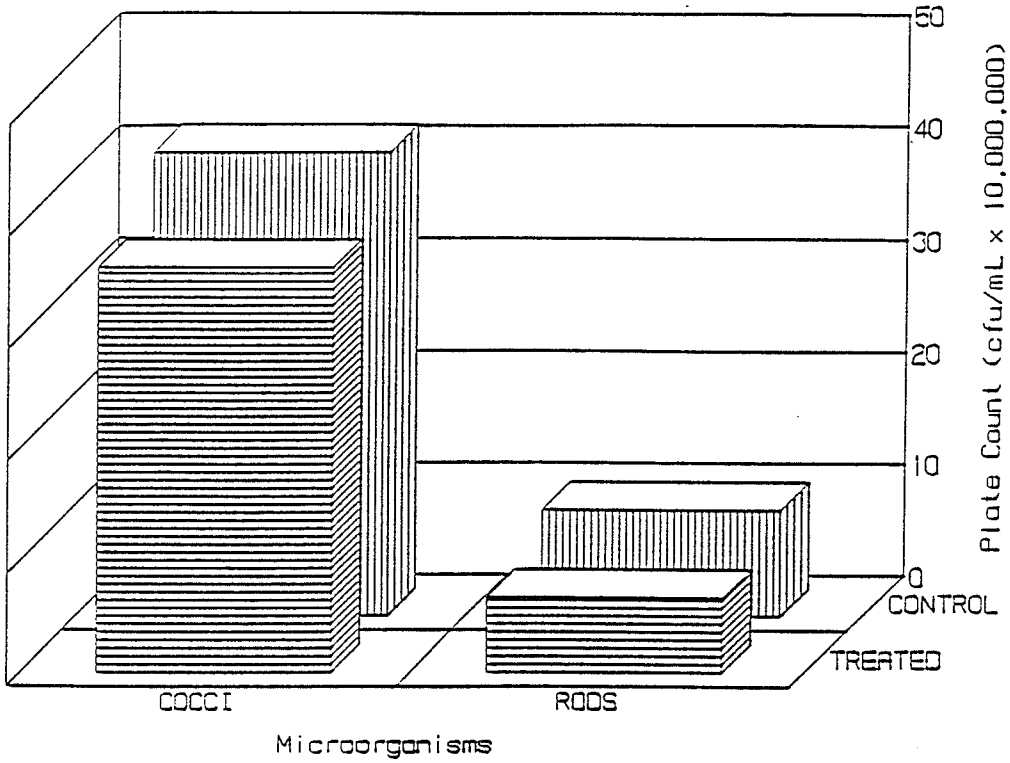
of 4.7%. A typical chromatogram is shown in Appendix 7. Concentrations of standard organic acids are given in Appendix 8.

5.2. Enumeration of Microorganisms

There was no difference in the total numbers of viable microorganisms between the control and treated cultures as determined by plate counts following 4 hr application of the 60 Hz, 4.3 G, 1.0% duty cycle, electromagnetic field. The control culture provided a mean microbial count of 4.00×10^8 CFU/mL, while the treated culture was enumerated at a mean value of 7.10×10^8 CFU/mL (Appendix 9, 10). No difference in the ratio of rods to cocci (Lactobacillus bulgaricus to Streptococcus thermophilus) was observed with differential agar between the control and treated cultures following enumeration with modified MRS medium (Figure 7, Appendix 11, 12). After 4 hr hours electromagnetic field exposure, control cultures had mean counts of 41.5×10^7 CFU/mL for S. thermophilus, while the yogurt culture exposed to the electromagnetic field (60 Hz, 4.3 G, 1.0% duty cycle) had mean counts of 36.2×10^7 CFU/mL. The control culture had mean counts of 9.5×10^7 CFU/mL for L. bulgaricus, while the culture treated with the 60 Hz electromagnetic field was enumerated at 6.7×10^7 CFU/mL.

Enumeration of microorganisms was also carried out on nutrient agar for yogurt culture enriched with calcium 5.0% above indigenous levels, following 4 hr exposure to the 60 Hz, 4.3 G, 1.0% duty cycle, electromagnetic field. Control (Ca^{2+}-enriched) cultures exhibited plate counts of 8.00×10^8

Figure 7. Distribution of cocci:rods (S.thermophilus: L.bulgaricus) expressed as mean values (CFU/mL); determined by plate count on modified MRS medium following 4 hr EMF: 60 Hz, 4.3 G, 1.0% d.c.



CFU/mL. The mean of plate counts on nutrient agar for calcium enriched yogurt culture gave counts of 4.83×10^8 CFU/mL (Appendix 13, 14). The plate counts from the calcium enriched and control cultures are effectively equitable, with no significant difference in total cellular population observed.

5.3. Temperature Monitoring and Control

Temperatures taken from tubes within the control and the two treatment coils were within 0.1° of one another and exhibited little variation with a sampling interval of 10 min (Figure 8). The mean temperature typically cycled between 41.7°C and 42.8°C. Typical incubator temperatures for yogurt culture in control and treatment coils over 240 min incubation are given in Appendix 15. Typical mean values for temperature measurements of yogurt culture in control and treatment coils are reported in Table 12. The mean value for typical temperatures in both the control and treatment coils was determined to be 42.3°C.

The effect of extraneous thermal energy on the development of the yogurt culture was assessed through enumeration of yogurt culture by plate count on nutrient agar following incubation at 42°C and 43°C (Appendix 16). The additional thermal energy (1.0°C) did not alter the number of viable microorganisms during the 4 hr incubation. Plate counts on nutrient agar (42°C, 48 or 72 hours) were 2.75×10^8 CFU/mL and 2.78×10^7 CFU/mL (43°C, 48 or 72 hr).

Figure 8. Measured temperature in EMF incubator as a function of incubation time (min). Measurements taken using thermocouple probes submerged in active yogurt culture in control and treatment coils.

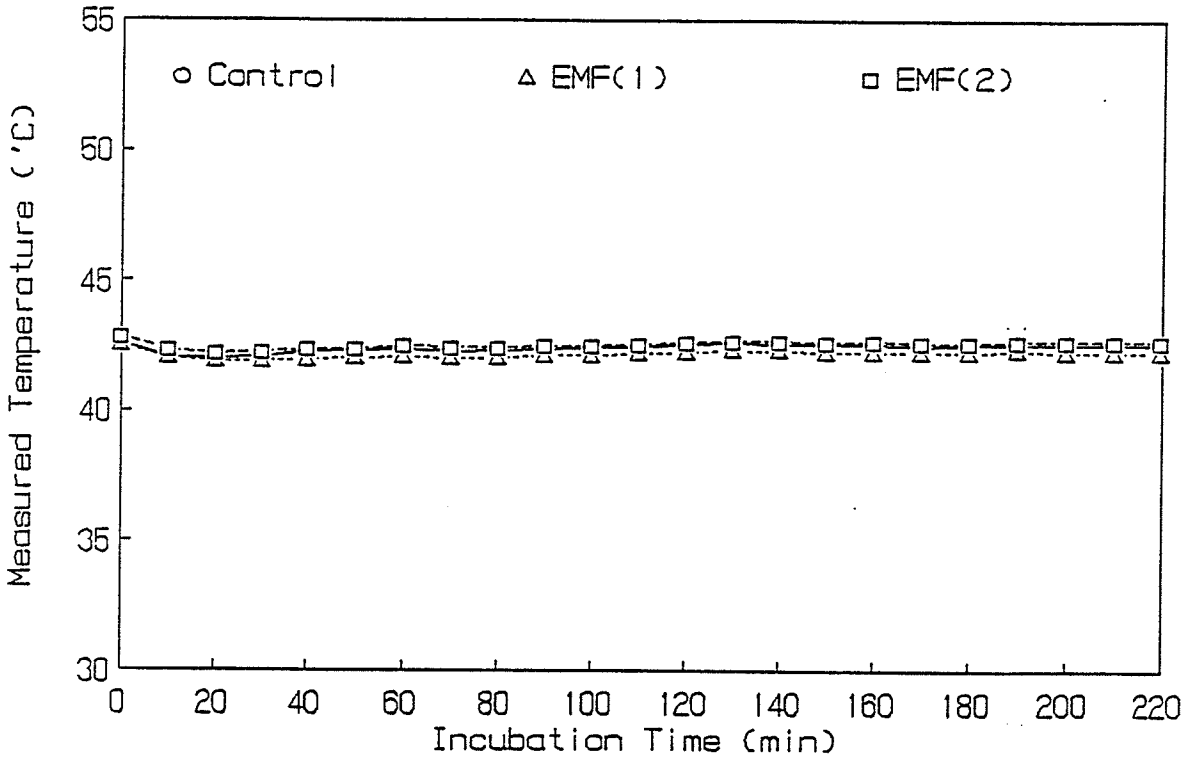


TABLE 12. Typical temperatures measured in control and treatment coils in incubator, mean values.*

Measured Temperature (°C)		
Control	EMF(1)	EMF(2)
42.29 ± 0.18	42.25 ± 0.18	42.27 ± 0.08

* (based on 240 min of samplings)

5.4. Results of Preliminary EMF Investigations

The electromagnetic field that caused the greatest biological activity had a field strength 4.3 G, a frequency of 60 Hz, a 1.0% duty cycle, and a square waveshape. No apparent effect was observed from the application of a 30 Hz, 4.3 G, 1.0% duty cycle electromagnetic field to spread plates of Pseudomonas fluorescens incubated at 7°C, although a slight stimulation in the development of colony forming units resulted from the application of a similar EMF with a frequency component of 60 Hz. Application of a 30 Hz, 4.3 G, 1.0% duty cycle electromagnetic field to spread plates of Bacillus stearothermophilus incubated at 25°C resulted in no apparent change in the development of colonies, unlike the 60 Hz, 4.3 G, 1.0% duty cycle EMF which resulted in a slight stimulation in the development of colony forming units. Application of a 60 Hz, 4.3 G, 1.0% duty cycle electromagnetic field to liquid cultures of Escherichia coli resulted in a slight inhibition of culture development, while a similar EMF with a frequency component of 600 Hz had no apparent effect on culture development. Cultures of the fungus Penicillium balaji exposed to an electromagnetic field of 60 Hz, 4.3 G, 1.0% duty cycle, exhibited a slight stimulation of culture development. Application of an electromagnetic field defined by a frequency of 60 Hz, 4.3 G, 1.0% duty cycle to a culture of Saccharomyces cereviseae in

0.1% peptone resulted in no apparent effect. Plate counts of control and treated cultures were 3.53×10^8 and 3.13×10^8 CFU/mL respectively, following 7 days of EMF exposure.

Detailed results of the preliminary electromagnetic field investigations on Ps. fluorescens, B. stearothermophilus, E. coli, P. balaji and S. cereviseae are reported in Appendices 17-22. A summary of the results of the preliminary EMF experiments is presented in Table 13.

TABLE 13. Summary of preliminary electromagnetic field investigations.

Organism	EMF	Temp. (°C)	Effect
Ps. fluorescens	30 Hz; 4.3 G; 1% d.c.;	7°C	_____
Ps. fluorescens	60 Hz; 4.3 G; 1% d.c.;	7°C	sl. stimulation
B. stearothermophilus	30 Hz; 4.3 G; 1% d.c.;	25°C	_____
B. stearothermophilus	60 Hz; 4.3 G; 1% d.c.;	25°C	sl. stimulation
E. coli	60 Hz; 4.3 G; 1% d.c.;	25°C	sl. inhibition
E. coli	600 Hz; 4.3 G; 1% d.c.;	25°C	_____
P. balaji	60 Hz; 4.3 G; 1% d.c.;	25°C	sl. stimulation
S. cereviseae	60 Hz; 4.3 G; 1% d.c.;	25°C	_____

6. DISCUSSION

Preliminary but extensive electromagnetic field investigations were carried out during the design/development stages of this investigation. These studies were intended to assess both the effectiveness of the developing electromagnetic field generating apparatus as well as determine a biologically effective range of magnetic field conditions. These tests involved exposing a variety of microorganisms (Ps. fluorescens, B. stearothermophilus, P. balaji, E. coli, and S. cereviseae) to electromagnetic fields with frequencies of 30, 60, and/or 600 Hz. The species-sensitivity and frequency-dependent nature of biological effects of electromagnetic fields were exemplified by the results of the preliminary studies.

Several preliminary experiments demonstrated the importance of frequency selection in the mapping of electromagnetic field response windows. Since the application of an electromagnetic field, characterized by a frequency of 60 Hz, to cultures of Ps.fluorescens, B.stearothermophilus and E.coli, and the fungus P.balaji resulted in alterations in metabolism, a frequency component of 60 Hz was selected for application to the yogurt culture.

Pulse duration and field strength were arbitrarily

established, based on successful results of the preliminary investigations. Pulse duration was established as a 1.0% duty cycle, in order to apply a minimal EMF pulsed signal to the developing yogurt cultures. Field strength was set at a level of 4.3 G, selected for being above the natural background of magnetic fields and for the ability of the power supply to maintain a magnetic signal at this level for extended periods. A square waveshape was established in order to minimize ramping (attack and decay) effects which may have further affected microbial development.

The selection of electromagnetic field parameters and the existence of electromagnetic field response windows in biological systems should be considered in the context of electromagnetic fields present in the natural environment. The largest naturally-occurring magnetic fields develop as the result of intense solar activity or thunderstorms: these electromagnetic fields are generally less than 0.00005 T with resonant magnetic frequencies of less than 40 Hz (Postow, 1987). Furthermore, naturally-occurring magnetic fields are generally of such low amplitude that there would be no evolutionary impetus for biological systems to develop protection against magnetic phenomena. It is reasonable to consider that biological

intra-cellular communication signals might therefore be expected to have evolved in a range of parameters (frequency, intensity, etc.) free from interference from the background of naturally-occurring electromagnetic field signals. In contrast, man-made electromagnetic fields (incidental or applied) are often of far greater magnitude than naturally-occurring electromagnetic fields. Man-made electromagnetic fields may typically range from 0.005-25 G (Postow, 1987). It is these electromagnetic fields which fluctuate from the background of naturally-occurring electromagnetic fields (elevated field strength, amplitude, etc.) which often affect the metabolism of biological systems.

Through the application of an electromagnetic field of frequency and amplitude which exceeds most naturally occurring EMFs, a measurable physiological response was observed in an actively growing lactic acid culture. The electromagnetic field was described in terms of frequency, magnetic signal amplitude, waveshape and pulse duration. The electromagnetic field response window with maximal effect on the lactic acid culture was characterized as possessing frequency of 60 Hz, a measured electromagnetic field intensity of 4.3 G, a duty (on/off) cycle of 1.0%, and square waveform. The effect of applying this electromagnetic

field to the lactic acid culture was an elevation in the production of certain organic acid(s) (specifically lactic acid), resulting in a slight decrease in the time required for yogurt coagulation under the experimental conditions.

The development of the yogurt culture occurs in two stages (Robinson, 1990; Tamine and Robinson, 1985). During the initial 4 hr of incubation Streptococcus thermophilus enters the exponential phase of growth resulting in the production of lactic acid, while the remainder of the fermentation is dominated by Lactobacillus bulgaricus which produces metabolites that affect the final flavor and aroma of the yogurt (Robinson, 1990). Since the EMF exposure in this research was conducted during the initial four hours of the yogurt fermentation (the period dominated by S. thermophilus) it was expected that alterations in the metabolism of the lactic acid culture would appear as variations in the levels of lactic acid.

From the experimental data a significant increase in titratable acidity was observed for yogurt cultures exposed to the 60 Hz electromagnetic field (1.0% duty cycle, 4.3 G). The total increase in titrated acidity in the culture exposed to the 60 Hz, 4.3 G, 1.0% duty cycle electromagnetic field was approximately 10% above that of the control

cultures. There were two distinct scenarios which would have accounted for this increase in titrated acidity: a direct increase in the population of S. thermophilus (with a corresponding elevation in titrated acidity), or an acceleration in the normal metabolic processes of the S. thermophilus population (also resulting in an elevation in titrated acidity).

In order to determine the cause of the increase in titratable acidity in yogurt cultures exposed to the electromagnetic field the microbial populations were enumerated by plate count methodology. Enumeration of microorganisms indicated there was no difference in the number of viable microorganisms between the cultures exposed to the 60 Hz, 4.3 G, 1.0% duty cycle, electromagnetic field and unexposed control cultures. Following 4 hr incubation and exposure to the electromagnetic field(s) mean plate counts were 4.00×10^8 CFU/mL, and 7.10×10^8 CFU/mL for the control and treated cultures, respectively. Since the total numbers of microorganisms (CFU/mL) in the control and treated samples are not significantly different, it appears likely the elevation of titrated acidity induced by the electromagnetic field was the result of an alteration(s) in the metabolism of S. thermophilus, rather than changes in the numbers of the microorganisms involved in the fermentation.

An increase in metabolic activity, and hence an increase in metabolic by-products within a constant time period has many positive implications for products (e.g. antibiotics, enzymes) produced by microbial fermentation methods.

High performance liquid chromatography was used for the analysis of organic acid concentrations in control and EMF-exposed yogurt cultures. The HPLC analysis of the organic acids was utilized as a means of monitoring trends in organic acid development, rather than for the comparison of specific concentration values (mg/mL), since the daily relative concentration of organic acids was a function of the vigor of the inoculating culture, which exhibited slight variation on a daily basis. Therefore, in order to compare trends in organic acid concentration, a determination of percent change in concentration of organic acids was used (Ross, 1990b).

High performance liquid chromatography analysis of organic acid levels in the yogurt culture determined the increase in titrated acidity of the EMF-treated cultures was the result of changes in the levels of organic acids present in the culture, although significant changes in concentration were observed only for lactic acid. No significant changes

were observed for the remaining organic acids (citric, orotic, pyruvic, acetic and propionic) in yogurt cultures exposed to the electromagnetic fields.

Manipulation of the frequency of the applied electromagnetic field demonstrated the frequency-dependent nature of the electromagnetic field response window. The frequency-dependent nature of the electromagnetic field response window was demonstrated in the variations of lactic acid concentration in the yogurt culture exposed to electromagnetic field frequencies of 50, 60 or 70 Hz. The greatest increase (5.9%) in lactic acid concentration occurred with the application of the 60 Hz, 4.3 G, 1.0% duty cycle electromagnetic field to the yogurt culture. Application of an electromagnetic field of 70 Hz, 4.3 G, 1.0% duty cycle resulted in a mean increase in lactic acid in the exposed population of 0.2%. Treatment of the yogurt culture with an electromagnetic field of 50 Hz, 4.3 Gauss, 1.0% duty cycle, caused a mean decrease in lactic acid of 1.3% below that of controls. This concurs with the results of Ross (1990b) and Postow (1987) who have described the importance of electromagnetic field frequency in the definition of EMF response windows.

In order to ensure that only the electromagnetic energy is responsible for the biological effects of an applied electromagnetic field, it is essential to maintain accurate and sensitive temperature monitoring. Thermocouple probes placed directly in tubes of actively developing yogurt culture provided precise measurement of in situ temperatures for both the control and treatment cultures. Thermal monitoring determined a uniform incubation temperature of 41.7-42.8°C for both the control and EMF-treated cultures. Variations in temperature resulting from the opening of the incubator to remove cultures resulted in slight alterations in temperature (1.0°C) which affected both control and treatment cultures equally, due to uniform air distribution from the circulatory fan. The effect of additional thermal energy (1.0°C) on the growth of the microbial population over the 4 hr incubation was examined. No significant increase in the numbers of viable microorganisms (CFU/mL) was observed between cultures incubated at 42°C and 43°C for 4 hr. This lack of change in the microbial population (CFU/mL) over the four hours exposure to the higher temperature (43°C) suggests that changes in the metabolism of yogurt culture during electromagnetic field exposure are not the result of incidental thermal energy, since the measured difference between in situ temperatures of the control and treated cultures did not exceed 0.40°C.

The mechanism(s) of the metabolic enhancement phenomenon on the development of the yogurt culture are not known, but since there are a large number of biological systems that can be affected by electromagnetic fields it is probable that more than a single mechanism is involved. One point of conjecture suggests a role for calcium in biological effects of electromagnetic fields. The function of calcium in biological effects of applied electromagnetic fields is not wholly understood, although numerous EMF-researchers have reported changes in calcium metabolism in biological systems exposed to electromagnetic fields (Lin-Xiang, 1990; Postow, 1987; Ross, 1990b; Yen-Patton, 1988; Zimmerman et al., 1990). Supplementing skim milk with calcium to an additional 5.0% that of indigenous level slightly decreased the metabolic enhancement phenomenon of the electromagnetic field on the yogurt culture. The calcium-enriched yogurt exhibited an increase in lactic acid levels of 4.7% that of control cultures following treatment with the 60 Hz, 4.3 G, 1.0% duty cycle electromagnetic field. The increase in lactic acid levels in the non-enriched culture was 5.9% that of controls, resulting in a mean difference in lactic acid production of 1.2%. This suppression of the metabolic enhancement phenomenon between calcium-enriched and non-enriched cultures suggests an active role for calcium in the mechanism of electromagnetic field

effects on yogurt development. It might be speculated that an abundance of extra-cellular calcium may have depressed the normal function of calcium channels in cells of S.thermophilus, resulting in decreased metabolic enhancement from the applied electromagnetic field.

The electromagnetic field of 60 Hz, 4.3 G, 1.0% duty cycle altered the metabolism of the lactic acid culture over the 4 hr exposure period. Application of the 60 Hz electromagnetic field to the yogurt culture resulted in an increase in titrated acidity of approximately 10%, which was attributed to changes in the metabolism of the culture. Application of similar electromagnetic fields, differing only in the frequency of the magnetic signal, resulted in different levels of metabolic enhancement of the yogurt culture. A 50 Hz electromagnetic field suppressed the metabolism of the yogurt culture over the four hour exposure, while a 70 Hz electromagnetic field exhibited little or no enhancement of the culture metabolism. Thus an electromagnetic field response window exists for the lactic acid culture, for an electromagnetic field characterized by a field strength of 4.3 G, a 1.0% duty cycle, and signal frequencies between 50-70 Hz. It is possible that other EMF

multi-parameter "response windows" exist, but these remain unmapped electronically. Serious consideration should be given to the short duration (4 hr) required for the electromagnetic field to affect the development of the yogurt culture. The fact that such relatively weak electromagnetic fields can induce alteration in biological systems over such a short time period should accentuate health considerations for more complex biological systems which are exposed daily to electromagnetic fields above the natural background EMF.

7. CONCLUSIONS

Lactic acid culture CH2 is utilized by the commercial dairy industry for lactic acid fermentations such as the production of yogurt. During this research program CH2 culture was exposed to a specifically defined electromagnetic field during the initial four hours of fermentation; the period dominated by the growth of S. thermophilus. An approximate ten percent elevation in acidity was observed in cultures exposed to a 60 Hz, 4.3 G, 1.0% duty cycle electromagnetic field. The increase in titrated acidity was attributed to an elevation in the concentration of lactic acid in the culture exposed to the electromagnetic field. The numbers of bacteria did not differ significantly between the control and treated cultures, suggesting alteration in cellular metabolism, (rather than an elevation in bacterial growth) was responsible for the increased acidity in yogurt cultures exposed to the electromagnetic field. The 60 Hz electromagnetic field response window provided the maximum enhancement of culture metabolism: electromagnetic fields of 50 Hz and 70 Hz resulted in minor changes to the lactic acid levels of -1.3% and 0.2%, respectively. Application of the effective 60 Hz electromagnetic field to yogurt culture supplemented with 5.0% calcium (over indigenous levels) decreased the metabolic enhancement phenomena compared to

cultures not supplemented with calcium. This suggests a role for calcium in the possible mechanism(s) of biological effects of these applied electromagnetic fields, possibly through alteration of calcium channels in cellular membranes. Overall it appears likely that more than a single mechanism would be involved in biological effects of electromagnetic fields considering the enormous range of biological systems which can be affected through the application of specific electromagnetic fields.

Potential applications for electromagnetic field technologies may lie in the area of selectively enhanced production of high value metabolic by-products of microbial fermentations. Selective enhancement of high value microbial metabolites could occur either as an increase in the rate of synthesis of metabolic by-products, or as an elevation in the final concentration of desired product(s).

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9. APPENDICES

Appendix 1.

Electromagnetic field generating systems:
power supply equipment employed by EMF researchers.

Author(s)	Apparatus
Moore (1979)	Kepco power supply (0-5V, 0-1A)
Ross (1990a)	custom designed amplifier; (control frequency and amplitude)
Goodman (1989)	Biosteogen system # 204
Lin-Xiang (1990)	Radioshack amplifier

Appendix 2.

Electromagnetic field generating systems: frequency generation and waveshape control apparatus employed by EMF researchers.

Author(s)	Apparatus
Moore (1979)	Wavetek function generator
Ross (1990a)	custom designed frequency control/amplifier
Goodman (1989)	Wavetek model 21 frequency generator
Lin-Xiang (1990)	Wavetek signal generator

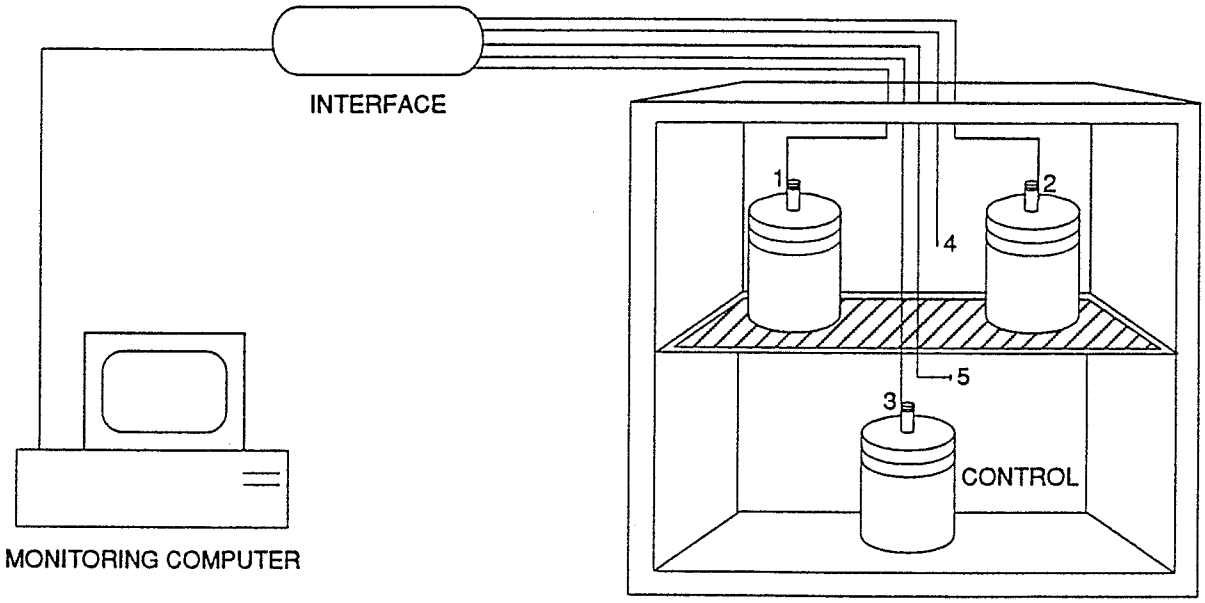
Appendix 3.

Electromagnetic field generating systems: magnetic field generating coils employed by EMF researchers.

Author(s)	Apparatus
Moore (1979)	silicone steel electromagnets; 1500 windings of 25 gauge insulated copper wire per pole
Ross (1990a)	helmholtz coil pairs; 70 windings of 18 gauge insulated copper wire per pole
Goodman (1989)	commercial helmholtz coils
Lin-Xiang (1990)	Electrobiology helmholtz coils

Appendix 4.

Thermal monitoring system in incubator. Thermocouples submerged in developing yogurt culture: 1-3. Incubator temperature thermocouples 4,5.



Appendix 5.

Change in pH of yogurt culture during exposure to EMF (60 Hz, 1% d.c., 4.3 G).

Time(hr)	Control	EMF(1)	EMF(2)
0	6.320^{a}	6.320^{a}	6.320^{a}
2	6.124^{b}	6.046^{b}	6.047^{b}
3	5.857^{c}	5.822^{c}	5.816^{c}
4	5.121^{d}	5.006^{d}	5.022^{d}

(Mean of three experiments;
(standard error = 0.053; $p<0.05$)

Appendix 6.

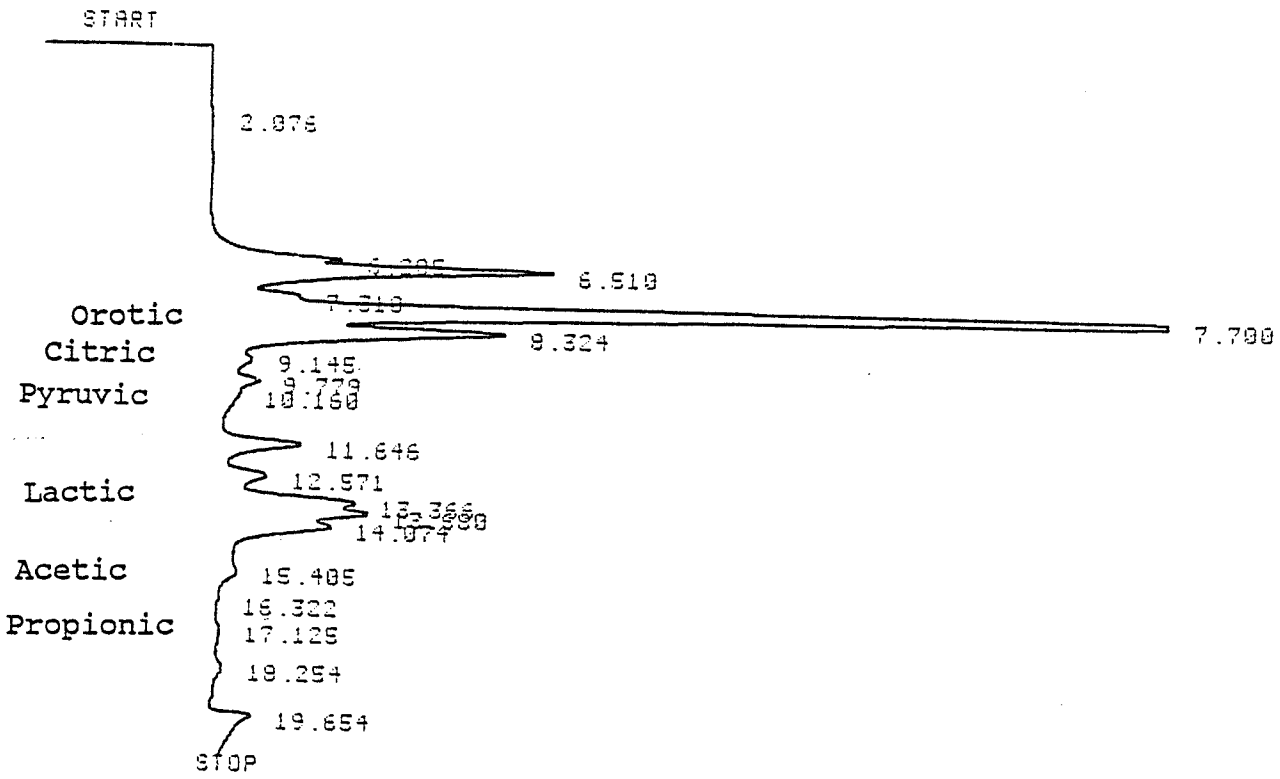
Change in titrated acidity over time during exposure to EMF (60 Hz, 1% d.c., 4.3 G).

Time(hr)	Control	EMF(1)	EMF(2)
0	2.33^{a}	2.33^{a}	2.33^{a}
2	2.30^{a}	2.50^{b}	2.50^{b}
3	2.97^{c}	3.23^{d}	3.30^{d}
4	5.13^{e}	5.57^{f}	5.60^{f}

(Mean of three experiments; standard error = 0.08; ($p<0.05$; Duncan's multiple range test)

Appendix 7.

Typical chromatogram of high perfomance liquid chromatography analysis of standard organic acids. Mobile phase: 0.006M H_2SO_4, column 69°C, UV detector set at 214nm. Organic acids include: lactic, orotic, acetic, propionic, pyruvic, and citric acid.



Appendix 8.

Concentrations of standard organic acids utilized for high performance liquid chromatography analysis.

Organic Acid	Concentration(mg/mL)
Lactic	3.4220
Orotic	0.0024
Acetic	0.0048
Propionic	0.0211
Pyruvic	0.0050
Citric	0.0140

Appendix 9.

Plate counts of yogurt culture on nutrient agar (CFU/mL) following exposure to EMF of: 60 Hz, 1% d.c., 4.3 G for 4 hr at 42°C. *

	CFU/mL		
Sample	Initial	Control	Treated
1	2.6×10^5 2.1×10^5	2.2×10^8 4.9×10^8	1.56×10^9 4.2×10^8
2	7.2×10^5 6.2×10^5	4.9×10^8 3.8×10^8	4.9×10^8 4.6×10^8
3	2.8×10^5 1.2×10^5	3.5×10^8 4.7×10^8	9.0×10^8 7.1×10^8
4	3.6×10^5 4.2×10^5	1.5×10^8 3.7×10^8	6.3×10^8 5.1×10^8

* (each value is the mean of duplicate platings)

Appendix 10.

Mean plate counts on nutrient agar following exposure to EMF of: 60 Hz, 4.3 G, 1.0% d.c. (48 hr; 42°C).

CFU/mL		
Initial	Control	Treated
$3.98 \pm 1.6 \times 10^5$	$4.00 \pm 1.0 \times 10^8$	$7.10 \pm 2.5 \times 10^8$

* (mean of quadruplicate samples)
** (each sample plated in duplicate)

Appendix 11.

Distribution of S.thermophilus and L.bulgaricus as determined by plate count on Modified MRS Agar, after 4 hr exposure to EMF: 60 Hz, 4.3 G, 1.0 % d.c. *

	CFU/mL	
	S.thermophilus	L.bulgaricus
Control	37.2×10^7	8.4×10^7
	52.0×10^7	9.1×10^7
	35.0×10^7	11.0×10^7
Treated	39.0×10^7	5.9×10^7
	21.5×10^7	4.3×10^7
	48.0×10^7	10.0×10^7

* (each value is the mean of duplicate platings)

Appendix 12.

Distribution of S.thermophilus and L.bulgaricus. Mean values of plate counts on modified MRS Agar; after 4 hr EMF treatment: (60 Hz, 4.3 G, 1.0% d.c.).

	CFU/mL x 10^7	
Culture	S.thermophilus	L.bulgaricus
Control	41.4 ± 7.1	9.5 ± 1.0
Treated	36.0 ± 9.7	6.7 ± 2.2

Appendix 13.

Plate counts of calcium enriched (5%) yogurt culture exposed to EMF: 60 Hz, 4.3 G, 1.0% d.c.

	CFU/mL*	
Sample	Control	5.0% Ca^{++}
1	1.9×10^9	6.2×10^8
2	2.6×10^8	9.3×10^8
3	4.1×10^8	2.0×10^8
4	6.3×10^8	1.8×10^8

* (each value is the mean of duplicate platings)

Appendix 14.

Mean values of plate counts of calcium enriched (5%) yogurt culture exposed to EMF: 60 Hz, 4.3 G, 1.0% d.c.

CFU/mL	
Control	Treated
$8.00 \pm 5.5 \times 10^8$	$4.83 \pm 2.9 \times 10^8$

Appendix 15.

Typical incubator temperatures during electromagnetic field application to yogurt culture. Temperatures measured by thermocouple probes submerged directly into yogurt culture (°C).

Time (min)	Control	EMF(1)	EMF(2)
0	42.02	42.97	42.34
15	42.01	41.96	42.15
30	42.09	41.96	42.15
45	42.25	42.02	42.21
60	43.30	42.24	42.37
75	42.38	42.24	42.37
90	42.27	42.32	42.41
105	42.34	42.28	42.33
120	42.29	42.32	42.28
135	42.36	42.30	42.25
150	42.26	42.34	42.24
165	42.19	42.27	42.18
180	42.11	42.15	42.12
195	42.08	42.12	42.07
210	42.02	42.05	42.02
225	42.00	42.04	41.98
240	41.97	42.01	41.96

Appendix 16.

Effect of temperature elevation on plate count (4 hr incubation), nutrient agar.

CFU/mL	
42°C	43°C
$8.3X10^7$	$1.4X10^8$
$4.1X10^8$	$6.1X10^7$
$2.8X10^8$	$3.2X10^8$
$3.3X10^8$	$5.9X10^8$
mean: $2.75 \pm 1.5 \times 10^8$	$2.78 \pm 1.8 \times 10^8$

Appendix 17.

Visual observation of Ps.fluorescens development at 7°C on tryptic soy plates. EMF variable frequency, 4.3 G, 1.0% d.c., and square waveform. Colony development indicated by: G (growth); SL.G (slight growth); or NG (no growth).

	30 Hz		60 Hz	
Day	Control	Treated	Control	Treated
1	NG	NG	NG	NG
2	NG	NG	NG	SL.G
3	SL.G	SL.G	SL.G	SL.G
4	SL.G	SL.G	SL.G	SL.G
5	G	G	G	G
6	G	G	G	G
7	G	G	G	G

(based on quadruplicate investigations)

Appendix 18.

Measured turbidity at 500-570 nm of Ps.fluorescens cultures exposed to EMF of 4.3 G, 1.0% d.c., 60 Hz, and square waveform.

Time (hr)	Control	Treated	% Change
0	0	0	0
0	0	0	0
24	150 ± 3.1	159 ± 4.4	+ 5.4%

(data represents mean value of 12 experiments)

Appendix 19.

Visual observation of B.stearothermophilus development at 25°C on tryptic soy plates. EMF: variable frequency, 4.3 G, 1.0% d.c., square waveform. Colony development indicated by: G (growth); SL.G (slight growth); or NG (no growth).

	30 Hz		60 Hz	
Day	Control	Treated	Control	Treated
1	NG	NG	NG	NG
2	NG	NG	NG	SL.G
3	G	G	G	G*
4	G	G	G	G
5	G	G	G	G
6	G	G	G	G
7	G	G	G	G

(based on quadruplicate investigations)
* (visually larger colonies observed)

Appendix 20.

Turbidity at 500-570 nm of E.coli cultures exposed to EMF of 4.3 G, 1.0% d.c., and 60 and 600 Hz at 25°C.

	60 Hz		600 Hz	
Time (hr)	Control	Treated	Control	Treated
0	0	0	0	0
6	0	0	0	0
24	150 ± 2.7	143 ± 3.5	150 ± 3.1	152 ± 5.4

(data represents mean values of 12 experiments)

Appendix 21.

Determination of effect of EMF on biomass of P.balaji following 7 days exposure to EMF of 4.3 G, 60 Hz, 1.0% d.c.*. Culture maintained at 25°C in ntrient broth.

Biomass (g)		
Control	Treated	Percent Change
0.091 ± .001	0.095 ± .001	+ 4.4 %

* (based on 14 investigations)

Appendix 22.

Effect of EMF on Saccharomyces cereviseae in liquid culture determined as CFU/mL, following 7 days exposure to EMF of 60 Hz; 4.3 G; 1.0% d.c. Culture maintained at 25°C in 0.1% peptone. Data represents mean value of quadruplicate experiments.

CFU/mL	
Control	Treated
$3.53 \pm 4.8 \times 10^8$	$3.13 \pm 1.6 \times 10^8$