B.S. Abdur Rahman Crescent Institute of Science & Technology
Deemed to be University u/s 3 of the UGC Act, 1956

17.3.2 c – Progress against SDG2

(i) Curriculum & Syllabus in the science behind plant biotechnology

(ii) Research Paper on "Bacterial inactivation in unpurified water"

B.S. Abdur Rahman Crescent Institute of Science & Technology

Deemed to be University u/s 3 of the UGC Act, 1956

Regulations 2017

Curriculum and Syllabi

(Amendments updated upto June 2020)

B.Tech.
(Biotechnology)

B.S. Abdur Rahman Crescent Institute of Science & Technology
Deemed to be University u/s 3 of the UGC Act, 1956

REGULATIONS 2017
CURRICULUM AND SYLLABI
(Amendments updated upto June 2020)

B.TECH.
BIOTECHNOLOGY

PROGRAMME ELECTIVES

Sl. No.	Course Group	Course Code	Course Title	L	T	P	C
1.	PE	BTCX01	Biophysics	3	0	0	3
2.	PE	BTCX02	Industrial Biotechnology	3	0	0	0
3.	PE	BTCX03	Bio-Organic Chemistry	3	0	0	3
4.	PE	BTCX04	Molecular Pathology	3	0	0	3
5.	PE	BTCX05	Food Biotechnology	3	0	0	3
6.	PE	BTCX06	Cancer Biology	3	0	0	3
7.	PE	BTCX07	Tissue Engineering	3	0	0	3
8.	PE	BTCX08	Developmental Biology	3	0	0	3
9.		BTCX09	Bioseparation Technology				
10.	PE	BTCX10	Proteomics & Genomics	3	0	0	3
11.	PE	BTCX11	Biomedical Instrumentation	3	0	0	3
12.	PE	BTCX12	Pharmaceutical Biotechnology	3	0	0	3
13.	PE	BTCX13	Medical Biotechnology	3	0	0	3
14.	PE	BTCX14	Drug Design and Development	3	0	0	3
15.	PE	BTCX15	Intellectual Property Rights	3	0	0	3
16.	PE	BTCX16	Recombinant DNA Technology	3	0	0	3
17.	PE	BTCX17	Material science	3	0	0	3
18.	PE	BTCX18	Molecular & Cellular Diagnostics	3	0	0	3
19.	PE	BTCX19	Biomedical Engineering	3	0	0	3
20.	PE	BTCX20	Biosafety and Bioethics	3	0	0	3
21.	PE	BTCX21	Healthcare Biotechnology	3	0	0	3

6.	CHCX06	Fundamentals of Physical Chemistry	2	0	2	3
7.	CHCX07	Green Technology	2	0	2	3
8.	CHCX08	Organic Chemistry of Biomolecules	2	0	2	3
9.	CHCX09	Polymer Science and Technology	2	0	2	3

Humanities Elective I

(to be offered in III Semester)

Sl. No.	Course Code	Course Title	L	T	P	C
1.	SSCX01	Fundamentals of Economics	2	0	0	2
2.	SSCX02	Principles of Sociology	2	0	0	2
3.	SSCX03	Sociology of Indian Society	2	0	0	2

Humanities Elective II

(to be offered in IV Semester)

Sl. No.	Course Code	Course Title	L	T	P	C
1.	SSCX04	Economics of Sustainable Development	2	0	0	2
2.	SSCX05	Industrial Sociology	2	0	0	2
3.	SSCX06	Law for Engineers	2	0	0	2

General Elective
Group I Courses
(To be offered in V semester)

Sl. No.	Course Code	Course Title	Offering Department
1.	GECX 101	Disaster Management	Civil
2.	GECX 102	Total Quality Management	Mechanical
3.	GECX 103	Energy Studies	Mechanical
4.	GECX 104	Robotics	Mechanical
5.	GECX 105	Transport Management	Automobile
6.	GECX 106	Control Systems	EEE
7.	GECX 107	Introduction to VLSI Design	ECE
8.	GECX 108	Plant Engineering	EIE
9.	GECX 109	Network Security	CSE
10.	GECX 110	Knowledge management	CSE
11.	GECX 111	Cyber security	IT
12.	GECX 112	Genetic Engineering	LS
13.	GECX 113	Fundamentals of Project Management	CBS
14.	GECX 114	Operations Research	Mathematics
15.	GECX 115	Nano Technology	Physics / Chemistry
16.	GECX 116	Vehicle Maintenance	Automobile
17.	GECX 117	Fundamentals of Digital Image Processing	ECE

BTCX05 **FOOD BIOTECHNOLOGY**

L	T	P	C
3	0	0	3

OBJECTIVES:

The course aims to

- Provide a programme of education which can enable its graduates to enter a career in the food industry as technologists capable of ensuring the production and marketing of safe and quality foods.
- Provide a broadly based technological education whose graduates can also enter into employment in other sectors of the food chain, or related technical sectors, where they can apply their technological skills.
- Allow individuals to develop their capacity to undertake research into problems relating to the production and marketing of safe and quality foods.

MODULE I INTRODUCTION 8

History of Microorganisms in food, Historical Developments, Taxonomy, role and significance of microorganisms in foods. Intrinsic and Extrinsic Parameters of Foods that affect microbial growth, Microorganisms in fresh meats and poultry, processed meats, seafood's, fermented and fermented dairy products and miscellaneous food products, Starter cultures, cheeses, beer, wine and distilled spirits, SCP, medical foods, probiotics and health benefits of fermented milk and foods products.

MODULE II PRIMARY & SECONDARY FERMENTATION 8

Brewing malting, mashing, hops, primary & secondary fermentation: Biotechnological improvements: catabolic repression, High gravity brewing, B-glucan problem, getting rid of diacetyl. Beer, wine and distilled spirits.

MODULE III FOOD QUALITY PARAMETERS 8

Emerging processing and preservation technologies for milk and dairy product, Microbiological Examination of surfaces, Air Sampling, Metabolically Injured Organisms,Enumeration and Detection of Food-borne Organisms. Bioassay and related Methods

MODULE IV FOOD PRESERVATION 7

Food Preservation Using Irradiation, Characteristics of Radiations of Interest, in

Food Preservation. Principles Underlying the Destruction of Microorganisms by Irradiation, Processing of Foods for Irradiation, Application of Radiation, Radappertization, Radicidation, and Radurization of Foods Legal Status of Food Irradiation, Effect of Irradiation of Food constituents

MODULE V STORAGE 7

Stability Food Preservation with Low Temperatures, Food Preservation with HighTemperatures, Preservation of Foods by Drying, Indicator and Food-borne Pathogens, Other Proven and Suspected Food-borne Pathogens.

MODULE VI FOOD QUALITY AND CONTROL 7

Analysis of food, major ingredients present in different product, Food additives colour, flavour, vitamins, Microbial safety of food products, Chemical safety of food products, heavy metal, fungal toxins, pesticide and herbicide contamination.

Total Hours – 45

TEXT BOOKS:

1. Modern Food Micro-Biology by James M. Jay, (2000), 6th edition, An Aspen Publication, Maryland, USA.
2. Food Microbiology: Fundamentals and frontiers by M.P. Doyle, L.R. Beuchat and Thoma J. Montville, (2001), 2nd edition, ASM press, USA.
3. Food Science and Food Biotechnology by G.F.G. Lopez & G.V.B. Canovas (2003), CRCPress, Florida, USA

OUTCOMES:

At the end of the course students will be able to

- Integrate the scientific disciplines relevant to food
- Apply and communicate technological knowledge to meet the needs of industry and the consumer for the production and marketing of safe and quality foods.

BTCX07	TISSUE ENGINEERING	L	T	P	C
		3	0	0	3

OBJECTIVES:

Students shall know about

- Basic concept of types of tissues, cell migration and therapeutic importance of tissue engineering
- Different aspects of cell culture and 3 dimensional cell culture
- Importance of growth factors, hormones and signalling method
- Scaffold synthesis and its application in tissue engineering
- Case studies and regulatory issues

MODULE I INTRODUCTION 9

Basic definition, Structural and organization of tissues: Epithelial, connective; vascularity and angiogenesis, basic wound healing, cell migration, current scope of development and use in therapeutic and in-vitro testing.

MODULE II CELL-CELL COMMUNICATION and IN VITRO CULTURE 9

Different cell types, progenitor cells and cell differentiations, different kind of matrix, cell-cell interaction. Aspect of cell culture: cell expansion, cell transfer, cell storage and cell characterization,3-D cell culture, Bioreactors.

MODULE III MOLECULAR BIOLOGY ASPECTS 9

Cell signaling molecules, growth factors, hormone and growth factor signaling, growth factor delivery in tissue engineering, cell attachment: differential cell adhesion, receptor-ligand binding, and Cell surface markers.

MODULE IV SCAFFOLD AND TRANSPLANT- SYNTHESIS and APPLICATION 9

Engineering biomaterials for tissue engineering, Degradable materials (collagen, silk and polylactic acid), porosity, mechanical strength, 3-D architecture and cell incorporation. Engineering tissues for replacing bone, cartilage, tendons, ligaments, skin and liver. Basic transplant immunology, stems cells: introduction, hematopoiesis.

MODULE V CASE STUDY AND REGULATORY ISSUES 9

Case study of multiple approaches: cell transplantation for liver, cardiovascular, neural, fetal tissue engineering and artificial womb, prosthetics. Ethical, FDA and regulatory issues of tissue engineering.

Total Hours –45

TEXT BOOKS:

1. Lanza, Langer and Vacanti(eds). Principles of Tissue engineering. Academic Press, 2nd Edition 1999
2. Minoth, Strehl, Schumacher. Introduction to Tissue engineering. Wiley VCH., 3rd Edition, 2005

REFERENCES:

related research papers

OUTCOMES:

Students shall be able to

- understand fundamentals of tissue engineering
- understand cell-cell communication and cell culture techniques
- understand how cell signaling molecules help in cell proliferation
- understand and apply the knowledge of scaffold synthesis and tissue engineering application
- apply to concept to different tissue engineering applications and will know the ethical and regulatory issue

CHCX07 **GREEN TECHNOLOGY** **L T P C**
2 0 2 3

OBJECTIVES

To make students conversant with the

- basic principles of green chemistry and green technology.
- wastes that causes hazards to human health
- chemicals that harms our environment
- need for green processes in various industries

MODULE I GREEN CHEMISTRY PROTOCOL 7

Need – Significance – 12 Principles with examples – R4 model – Life cycle analysis – sustainable and cleaner production - Green Technology: definition, examples: CFC free refrigerants, green building, energy, 3D printers, nanotechnology – Awards for Green chemistry – organization promoting green chemistry.

MODULE II WASTE & WASTE MINIMISATION 8

Source of wastes: domestic, industrial, medical, nuclear, e-waste; problems; prevention – economy of waste disposal – Waste minimization techniques: general waste treatment and recycling – alternate waste water treatment technologies: hybrid process – Green computing: goals, green cloud, green ICT - Pollution statistics from various industries (Industrial case studies).

MODULE III GREEN SYNTHESIS 7

Introduction - Solvent free reactions - green reagents, green solvents in synthesis - microwave and ultrasound assisted reactions – supercritical fluid extraction – green oxidation and photochemical reactions – catalyst and biocatalysts.

MODULE IV GREEN INDUSTRIAL PROCESSES 8

Polymer industry: biodegradable polymer - textile industry: greener approaches of dyeing, waste disposal – ecofriendly agrochemicals: biofertilizers, biopesticides – Pharmaceutical industry: atom economy, reduction of toxicity, use of biocatalyst, zero waste disposal – Leather industry: greener process in tanning, crusting, surface coating – ecofriendly batteries & fuel cells.

L:30 periods

PRACTICALS

1. Synthesis of an ionic liquids (Ex: imidazolium) and testing the solubility of organic chemicals.
2. Green bromination of stilbene (using pyridine hydrobromide).
3. Green synthesis: Photocatalytic reactions, solvent-free organic reaction – Aldol; green oxidation, green reduction.
4. Microwave assisted chemical reaction. (synthesis of aspirin, pinacol-pinacolone reaction, etc).
5. Comparison of conventional reaction with microwave assisted reactions (atom economy, solvent, etc) [Ex: aldehyde and ketones with hydrazines to give hydrazones].
6. Diels-Alder reaction in eucalyptus oil (green process).

P:30 periods

Total: 60 periods

REFERENCES

1. Jain P.C and Renuka Jain, Physical Chemistry for Engineers, Dhanpat Rai and Sons, New Delhi. 2001.
2. V. K. Ahluwalia, Green Chemistry: Environmentally Benign Reactions, Ane Books India, New Delhi, 2006.
3. Paul Anastas, John C.Warner, John Warner Joint; Green Chemistry: Theory & Practice New Ed Edition; Oxford University press, USA, 2000.
4. Rashmi Sanghi, M. M. Srivastava, Green chemistry, Narosa publishers, New Delhi, 2003.

OUTCOMES

The students will be able to

- outline the principles and implications of green chemistry.
- comprehend the potential risks of waste generated and analyse the threats to human and environment.
- integrate information into design of molecules to avoid/eliminate toxic solvents & reagents or reduce toxic products.
- identify various alternate greener technologies for various industries.

GECX 108	PLANT ENGINEERING	L	T	P	C
		3	0	0	3

OBJECTIVES:

- To provide in depth knowledge on Plant Engineering
- To introduce detail engineering and P&ID
- To learn about the support to Instrumentation from other disciplines
- To study about the Installation and commissioning

MODULE I INTRODUCTION OF PLANTS 7

General Project Cycle – Feed – Sales - Plant Description, Component / Areas of Plant, Plant Layout, Plant Interfaces, Plant Location

MODULE II ELEMENTS OF PLANT 8

Main Elements of a Plant, Process Flow Scheme (PFD – Process Flow Diagram) P&ID's, Plant Legend Finalization.

MODULE III DETAIL ENGINEERING 10

P& ID Development with PFD's, Major Discipline Involvement & Inter discipline Interaction, Major Instrumentation & Control Systems - Development Phase – Instrument List , I/O Count, Specification Sheets, Instrument Installation (Hook ups) , Control Philosophy – Detail Engineering.

MODULE IV SUPPORT FROM OTHER DISCIPLINE 8

Other Discipline Supports to Instrumentation – Plot Plan, Piping / Equipment Plan, Electrical Area Classification, Fire Hazardous Classification Telecommunication Systems - Control Network architecture.

MODULE V INSTALLATION AND COMMISSIONING 7

Plant Construction - Key Drawings for Construction Support Construction Activities, System Testing, Startup / Commissioning, Production.

MODULE VI CASE STUDIES 5

Case studies of Water Treatment Plant - Paper Industry – Power Plant etc

L – 45; Total Hours –45

REFERENCES:

1. Duncan C Richardson, Plant Equipment and Maintenance Engineering Handbook, McGraw-Hill Education: New York, Chicago, San Francisco, Athens, London, Madrid, Mexico City, Milan, New Delhi, Singapore, Sydney, Toronto,2014 McGraw-Hill Education
2. Gabriel Salvendy, Handbook of Industrial Engineering – Technology and operations Management, John Wiley & Sons, 2001.
3. Robert C Rosaler , Standard Handbook of Plant Engineering, Mc Graw Hill third Edition, 2004
4. R. Keith Mobley, Plant Engineer's Handbook, Technology and Engineering, 2001.

OUTCOMES:

At the end of the course, the student will be able to

- Review and correct P&IDs
- Do installation and commissioning of new plants
- Apply plant engineering in design and maintenance of water treatment plant / power plant etc

Japan Journal of Food Engineering, Vol. 20, No. 2, pp. 63 – 70, Jun. 2019 DOI : 10.11301/jsfe.19546

◇◇◇ Technical Paper ◇◇◇

High Voltage Pulsed Electric Field Application Using Titanium Electrodes for Bacterial Inactivation in Unpurified Water

Ramya RAMASWAMY[1,†], Raja PRABU RAMACHANDRAN[2], V. GOWRISREE[3]

[1]*Research Scholar, Department of Electrical and Electronics Engineering, BS Abdur Rahman Crescent Institute of Science and Technology, Seethakathi Estate, Vandalur, Chennai – 600048, India.*
[2]*Professor, Department of Electrical and Electronics Engineering, BS Abdur Rahman Crescent Institute of Science and Technology, Seethakathi Estate, Vandalur, Chennai – 600048, India.*
[3]*Professor, Department of Electrical and Electronics Engineering, College of Engineering, Anna University, Guindy, Chennai – 600025, India.*

Pulsed Electric Field (PEF) treatment is considered as nonthermal due to application of ultra short high voltage pulses in liquid foods to extend their shelf life. In today's world, water decontamination becomes extremely necessary to safeguard people from health ailments. The objective of this work focusses on inactivation of naturally prevailing *Escherichia coli* and *Fecal coliform* bacteria in environmental water using titanium electrodes. In this study, the PEF treatment chamber was designed to be used for both static and continuous modes of treatment. Bipolar square wave pulses having 1 μs pulse width at a rise time of 160 ns and pulse repetition frequency between 48 to 50 Hz were used in this research. From the results, it was observed that titanium effectively inactivated both the microorganisms at a minimum treatment time of 60 seconds at 33.9℃ while conventional stainless steel required 120 seconds at a temperature of 40.1℃ under the same experimental conditions. Also, the relationship between treatment time and temperature remained linear despite the change in electric field. Results confirmed that (i) Titanium is more suitable in PEF for water decontamination due to its high reactivity than stainless steel (ii) Using titanium, complete ABSENCE of the two microorganisms could be possible in water at a nominal field strength of 24 kV/cm with much less temperature requirement.

Keywords: Pulsed Electric Field, Titanium Electrodes, Water Decontamination, *Escherichia coli* and *Coliform*.

1. Introduction

Pulsed Electric Field treatment (PEF) has a greater potential for inactivating viable pathogens present in liquid food products through electroporation [1–4]. The electric field can be applied in the form of ultra short high voltage pulses (20 kV/cm to 80 kV/cm) to liquid food present in PEF treatment chamber accompanying two electrodes. In previous PEF studies, stainless steel was generally recommended as the electrode material [1, 5–9]. Though other metals have also been used, the use of titanium is still limited in PEF and needs further growth in applications. So far, PEF has given successful inactivation rates in various fruit juices, but it's role on water decontamination is still not fully developed to make the technology a complete one. Water contamination is a major concern today, as the bacteria in water pose a greater health risk when consumed. In this work, water was chosen as the testing liquid because it is one of the basic needs for everyday consumption. If water is not properly treated and consumed, it can lead to stomach related diseases. Hence this work focusses on two major factors. (i) Electrode Material – In most of the PEF observations, stainless steel electrodes were used as the state of art material which has given effective inactivation rates. But when the corrosion properties of stainless steel were analyzed, the material reported more metal ion release than titanium [10,11]. When titanium is considered, it is a more durable metal which has been used extensively in surgical implants and in cooking utensils due to its nontoxic properties. It is less dense and has high strength than stainless steel. While stainless steel is basically an alloy made up of a mixture of chromium, iron and sometimes other metals to enhance its corrosion resistant property, titanium's characteristics are naturally found within it. Under fluctuating changes in temperature, titanium is a better choice than stainless steel

(Received 12 Mar. 2019: accepted 10 May. 2019)
† Fax: +919789963079, E-mail: ramya@crescent.education

because it is highly resistant to fatigue. Titanium is also more reactive than stainless steel because it reacts readily with oxygen and forms stable and protective oxide layer on the metal surface. Due to this oxide layer, it has excellent corrosion resistant property. Even when oxide layer gets damaged, it will reheal itself instantaneously if traces of oxygen or water is present in the environment [12]. The resistivity of titanium ranges from 4.2×10^{-7} to 5.2×10^{-7} ohm–m [13] whereas for stainless steel it is 7.4×10^{-7} ohm–m [13].

Previous studies used either uncoated or platinum coated titanium electrodes for electrolysis treatments including water ionizers, waste water electrolysis treatments, electrochemical and electrocoagulation treatments due to its excellent electrochemical stability [14–16]. These treatments rely on application of continuous electric current passing through the water for disinfection which may require high temperature and treatment time and also may result in higher concentration of metal ion release into the liquid medium but in PEF, electric current plays only a trivial role and the microbial inactivation depends on high voltage pulse application in microseconds. Hence, the methodology adopted and mode of application for all the above techniques are different when compared with PEF, other than the use of titanium electrodes as one of their electrode materials. Recent research highlighted the potential of titanium on higher retention of ascorbic acid than stainless steel under ohmic heating method [17]. While very few studies used titanium for PEF applications in liquid foods [18], neither of previous work has focused on reaction of titanium in water under PEF treatment. Hence, under this study titanium was chosen as the electrode material. (ii) Water decontamination – People in rural areas drink unpurified water from several sources. Hence it was proposed to decontaminate unpurified water using titanium electrodes under PEF treatment, targeting the most naturally surviving *Escherichia coli (E.coli)* and *Coliform* bacterial loads. Many water treatment methods are available today which are considered as effective including UV systems, Ozone water purification, chlorination and especially reverse osmosis (RO) water purifiers. These water treatment methods have their own disadvantages. For instance, while UV and ozone systems have the potential to induce carcinogenic effects, chlorination involves addition of chemicals. Though reverse osmosis water treatment is considered as a complete technology for water purification, it has the drawback of demineralizing the water and wasting 3–4 liters to purify one liter of water [19]. The wasted water also becomes non consumable. But PEF can effectively decontaminate water with the following advantages (i) No thermal effect (ii) No addition of chemicals (iii) No water wastage (iv) No hazardous effects on health (v) No demineralization.

2. Materials and Methods

2.1 High Voltage Pulse Generator

A high voltage pulse generator is used for delivering high electric field pulses to liquid food placed between two electrodes in PEF treatment chamber. Fig. 1 shows the circuit of high voltage pulse generator consisting of a 10 stage Pulse forming network (PFN). In this research, the pulse forming network is designed to deliver bipolar square waveforms having positive and negative polarities, where each polarity has a pulse width of around 1 μS. Basically, the pulse forming network comprises of high energy storage components such as capacitors, inductors, and transmission lines which can be charged by a high voltage DC power source, and then rapidly discharged into load (PEF treatment chamber).

In other words, the voltage from the supply is stepped up and rectified into high DC voltage and then is given in the form of pulses to the load through pulse forming network (PFN). A spark gap switch is employed between

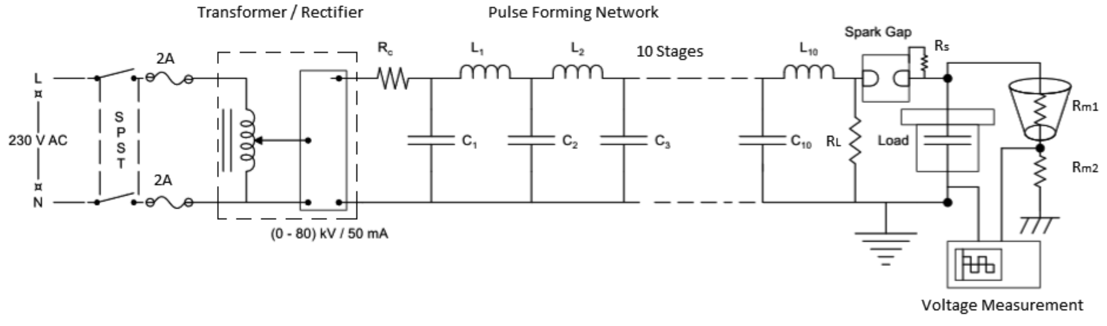

Fig. 1 Circuit of square wave pulse generator.

the PFN and load for switching high voltage pulsed power applications. A resistor R_s of 100 MΩ is connected across the spark gap switch to protect the device during breakdown or under dynamic high voltage conditions. Previous studies reported the efficiency of using square wave pulses for bacterial inactivation when compared with other waveshapes [5,20,21]. Bipolar pulses were chosen in this research due to better bacterial inactivation, reduction of electrolysis with undesirable temperature increase in liquid food [5,20,21].

Since pulse forming networks represent approximation for a transmission line, they must be charged up to twice the desired output pulse voltage since half of the voltage will be dropped across the PFN impedance and the remainder across the load impedance. Hence in this work, the voltage delivered to the load V_L will be half that of the voltage V_0 charged through the capacitor bank from C_1 to C_{10}. Here, V_0 is the no load charging voltage and V_L is the load voltage or the voltage across the electrodes. The value of capacitance for each charging capacitor ($C_1 - C_{10}$) is 1000 pF, resistance of charging resistor R_c, is 1 kΩ, Inductance value for each Inductor ($L_1 - L_{10}$) is 2.5 μH with a voltage rating of up to 100 kV are used in the PFN respectively. A resistor R_L with a resistance of 50 Ω is connected across the capacitor bank before the pulsed energy getting discharged to the load for impedance matching purposes. If the characteristic impedance of the PFN is matched to that of the load, the energy will be dissipated to the load without any further voltage drop or reflection, where the voltage across the load will be one half the charged voltage of the PFN capacitors as previously stated. Here, the charging voltage across the electrodes play a major role in generating the required electric field for bacterial inactivation. The current will be less significant in this study and the value will be negligible in few milliamps. For instance, in this study the resistance across two electrodes in water sample is measured as 9.5 MΩ. According to ohms law, when the charging voltage across the electrodes is 4 kV, the corresponding current drawn by them will be only 0.5 mA. Then the generated electric field is calculated theoretically as [22]

$$E=V/d \tag{1}$$

Where V is the charging voltage across electrodes and d is the distance gap between them. Thus, Inactivation of microbes can be successfully obtained using ultrashort high voltage pulse application under PEF treatment. We can observe the cursor measurement representing the total pulsewidth of a bipolar square waveshape having positive and negative polarities, each having a pulsewidth of around 1 μS applied across the electrodes in water as shown in Fig. 2.

The specifications for the pulsed input are shown in Table 1

2.2 Instrumentation

A 100 MΩ (R_{m1}) 1000X Tektronix voltage compensated probe is connected across the treatment chamber and Tektronix TDS 2022 oscilloscope (bandwidth of 200 MHz and peak sample rate of 2 GS/s) for high voltage measurements. Temperature of the water was monitored after the treatment using Instrumentics digital food thermometer which can measure food temperature from −50℃ to+300℃.

2.3 PEF Treatment Chamber

Parallel plate electrodes were used in this research as shown in Fig. 3, which can provide uniform electric field distribution in the treatment region. Previous studies used round parallel plate stainless steel electrodes due to the advantage of having simple geometrical dimensions and uniformly treated liquid in the treatment area [5]. In this research, the circular edges of electrodes were smoothened to avoid possible field fringing on those

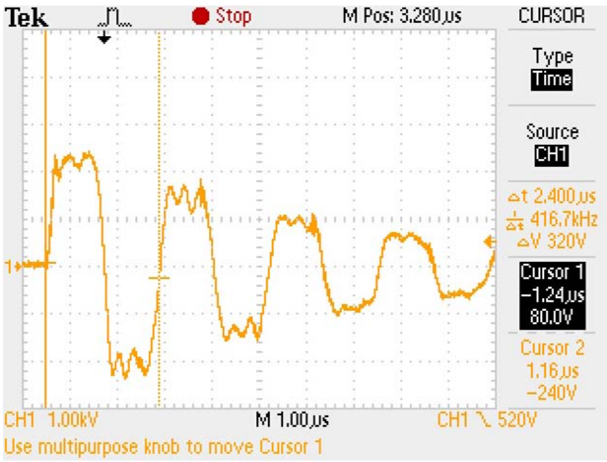

Fig. 2 Bipolar square waveform across electrodes in water sample.

Table 1 Specifications for pulse profile

Pulse Profile	Specification
Pulse Waveshape	Square
Polarity	Bipolar
Pulsewidth for each polarity	~1 μS
Pulse Repetition Frequency	(48 − 50) Hz

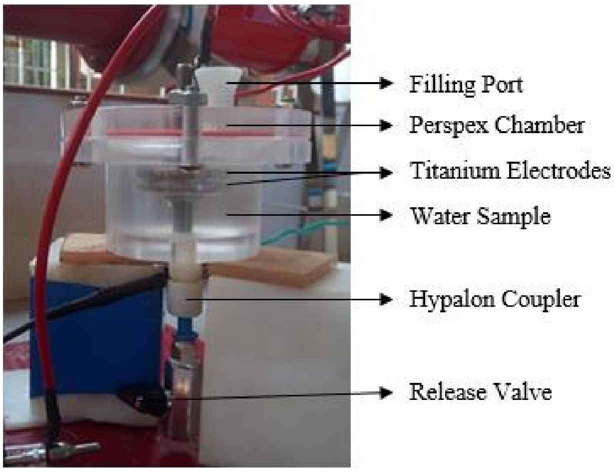

Fig. 3 PEF chamber with water sample.

edges. The PEF chamber is made up of autoclavable acrylic material (Perspex) with inlet or filling port and liquid release valve. The novelty lies in the chamber design, which can be used for both static and continuous modes of PEF treatment and also have a screw arrangement so that the distance gap d between two electrodes can be varied. The volume of PEF treatment chamber accompanies 120 ml of liquid to be tested. The diameter and thickness of the electrodes used were 40 mm and 5 mm respectively. The inner cylindrical volume has a diameter and height of 70 mm and 50 mm respectively.

2.4 Sterilization of Experimental Equipment

Before performing the experiment, the chamber was autoclaved, cleaned with warm soapy water followed by isopropyl alcohol and sterile water. The immersing section of food thermometer was disinfected with isopropyl alcohol before measurement.

2.5 Microbial Parameters

The microbial parameters chosen for inactivation were *E.coli* and *Fecal Coliform* bacteria, which are naturally present and predominantly surviving microorganisms in environmental and domestic tap water. The water sample was collected from a remote area in Karnataka from a public tap. The collecting vials were thoroughly sterilized before filling up with untreated water for maintaining accuracy. After confirming the presence of these two microorganisms in tap water, further analysis was initiated. The reference microorganism used for identifying the type of bacteria in water was *E.coli MTCC 433* which is a rod-shaped gram-negative bacterium. In other words, the identification of *Ecoli* was performed by comparing with standard *E.coli MTCC 433*. This microorganism can live in human intestines and can cause stomach related illness in humans [23]. *Coliforms* will be usually found in the environment, where feces of man and other warm-blooded animals will be present. The presence of *coliform* bacteria in environmental water may relate to the presence of harmful, disease causing microorganisms. For the microbial analysis, PRESENCE / ABSENCE method was adopted

2.6 Water sample analysis

Water sample was analyzed for *E.coli* and *Coliform* bacteria after every PEF application. While bacterial count was emphasized in previous research studies, complete absence of bacteria was primarily required in this research to ensure water quality and safety for everyday consumption.

2.6.1 Membrane Filtration Method

The Indian standard method IS 15185:2016 (International standard equivalent ISO 9308-1:2014) was adopted for evaluation and detection of *E.coli* and *coliform* bacteria in water samples after application of high electric field. This method is usually recommended for bacteriological examination of water. Under this method, the sample is passed through a membrane using a filter funnel and vacuum system as shown in Fig. 4a. The presence of microorganisms will be trapped on the membrane surface. This membrane with bacterial concentra-

a)

b)

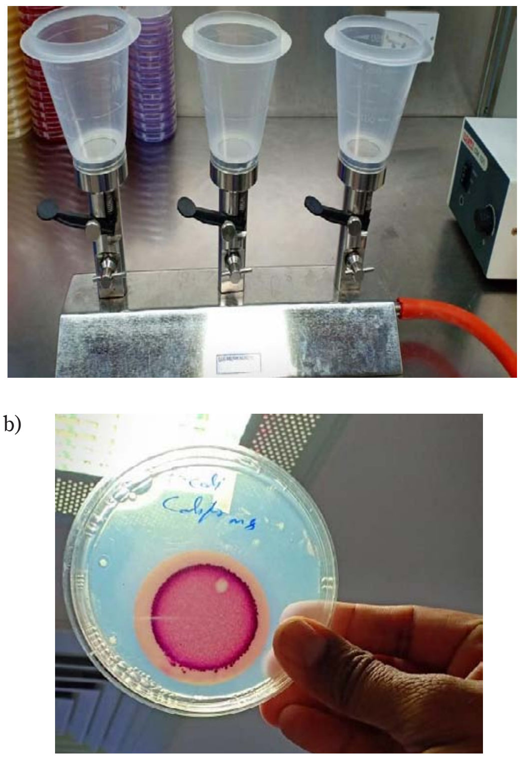

Fig. 4 a) Membrane filtration setup b) Escherichia coli and coliform analysis on petri dish.

tion will be placed in a special glass plate equipped with a pad saturated with appropriate growth medium. The passage of nutrients through the filter during incubation period enables the growth of microorganisms on the upper membrane surface as shown in Fig. 4b. The bacteria thus grown can be easily transferred to confirmation media. Membrane filter technique is thus an effective, accepted technique for testing fluid samples for microbiological contamination.

2.6.2 Significance of PRESENCE – ABSENCE (P/A) approach

IS 10500 is the most recommended Indian standard for indicating the limits in bacterial parameters in water. The result will be interpreted as PRESENT irrespective of any number of viable pathogens present in the water and will not be certified as safe for drinking purposes. The result will be interpreted as ABSENT for complete absence of bacteria which is primarily required, satisfying the objective of this research work. Hence according to IS 10500, *E.coli* and *Coliform* bacteria should be absent or not be detectable per 100 ml of water for certification as safe for everyday consumption. However, data on initial concentration was required for concise approach. Accordingly, the initial concentration of *E.coli* and *coliform* bacteria was found to be around 500 MPN and 700 MPN per 100 ml of untreated water, where MPN represents most probable number of bacterial densities found in 100 ml of water sample.

3. Results and Discussion

3.1 Impact of electric field and treatment time on microbial inactivation

Treatment time is expressed as multiplication of number of pulses by pulse width. Increasing either of the two will result in higher microbial inactivation. In this research, pulse width was made constant throughout the experiment and number of pulses were increased. According to previous studies, by maintaining a constant pulsewidth, the energy consumption can be reduced [24]. When the number of pulses increases, the associated treatment time will also increase. Hence, the treatment time was varied from 30 to 360 seconds for titanium electrodes and from 60 to 180 seconds for stainless steel electrodes. Since the research focus was on titanium electrodes, comparative study with stainless steel was made only with observations showing difference in the results. Here, the field was gradually increased to prevent sudden sparking between the two electrodes. The general range of electric field strength used in PEF treatment will be from 20 kV/cm to 80 kV/cm for getting good inactivation results. However, there are some studies which has given inactivation rates at lesser field values [25,26]. Since the novelty lies on the treatment chamber design, it was necessary to determine a suitable field value which should obtain effective inactivation rates using this chamber design. Hence, determining the field value was based on two important factors (i) Should be a nominal value, which should not induce thermal effects (ii) Should have inactivation effect on both *E.coli* and *Coliform* bacteria.

For the purpose, initial experimentation was carried at a distance gap of 5 mm, which generated electric field of 9.4 kV/cm at a treatment time of 30 seconds. Results reported that at this distance, the field generated was not sufficient to inactivate both the bacteria and the corresponding treatment time was found to be less effective on these microorganisms. It was also noted that visual effects of discharges indicating the reaction between the electrode and water could not be seen at 30 seconds. This initial observation aided in fixing the suitable field value and a starting treatment time for further applications. Accordingly, the distance gap was adjusted and reduced to 2 mm which generated a field strength of 24 kV/cm. At this field, the corresponding treatment time was set for 60 seconds. While the pulses were continuously applied, a notable reaction of visual discharges was observed at 24 kV/cm when the treatment time approached 60 seconds.

Under these conditions, both the bacteria were completely inactivated *i.e.*, a complete absence indicating zero bacteria at a temperature of 33.9℃ using titanium electrodes. The following inactivation results are shown in Table 2.

From the above results, the microbes were inactivated from 60 seconds onwards at a field strength of 24 kV/cm. Hence, this time was taken as a standard of comparison with stainless steel under the same experimental conditions. Using stainless steel, observations were made till 180 seconds as further increase in treatment time resulted in saturation and all the necessary results were obtained within the time course followed. The corresponding results are shown in Table 3.

In the case of stainless steel, while absence of *E.coli* and presence of *Coliform* was observed at 60 seconds, the metal took 120 seconds to inactivate both the microorganisms at a temperature of 40.1℃. Hence only half of the treatment time was taken by the titanium electrodes

Table 2 Inactivation results for Titanium Electrodes

Electrode Material	Electric Field (kV/cm)	Distance gap between electrodes (mm)	Treatment time (seconds)	(P/A)* per 100 ml of water sample	
				E coli	*Coliform*
Titanium	Zero Field (Untreated Water)	NA	NA	P	P
	9.4	5	30	P	P
	24	2	60	A	A
	24	2	90	A	A
	24	2	120	A	A
	24	2	150	A	A
	20	2	180	A	A
	24	2	240	A	A
	24	2	300	A	A
	24	2	360	A	A

*P/A=Presence/Absence of bacteria per 100 ml of testing water sample

Table 3 Inactivation results for stainless steel electrodes

Electrode Material	Electric Field (kV/cm)	Distance gap between electrodes (mm)	Treatment time (seconds)	(P/A)* per 100 ml of water sample	
				E coli	*Coliform*
Stainless Steel	Zero Field (Untreated Water)	NA	NA	P	P
	24 kV/cm	2	60	A	P
	24 kV/cm	2	90	A	P
	24 kV/cm	2	120	A	A
	24 kV/cm	2	150	A	A
	20 kV/cm	2	180	A	A

*P/A=Presence/Absence of bacteria per 100 ml of testing water sample

for inactivation.

3.2 Impact of electric field and treatment time on temperature increase

A complete ABSENCE of bacteria in water was obtained at 60 seconds and 120 seconds using titanium and stainless steel electrodes respectively. After getting the inactivation results, saturation effects started to emerge. Under this condition, the relationship between other parameters such as the treatment time, electric field and temperature was studied. While temperature control is an important parameter to be considered in PEF in fruit juices to retain the food's quality attributes, it is also significant in the case of water for reduced energy consumption than other heat treatments. Hence optimization of electrical parameters was required to get better inactivation in PEF at less temperature. The electric field and treatment time are the two important input parameters that will influence the temperature increase in liquid food. Under this study, the temperature was monitored for (i) Constant electric field of 24 kV/cm (ii) Sudden reduction of electric field from 24 kV/cm to 20 kV/cm at 180 seconds in the mid of experimentation. Under both circumstances, the temperature increased linearly for increase in treatment time. Though the field was reduced suddenly, the treatment time was still in increasing mode and hence temperature also increased. From the observations, it was understood that increase in treatment time had a primary impact on temperature increase than change in electric field. Thus, reduction in field did not influence the linear relationship between the treatment time and temperature. This study confirmed that the chosen field value was in nominal range. The linearity can be observed in the graphical data for titanium and stainless steel as shown in Fig. 5a and 5b respectively.

a)

b)

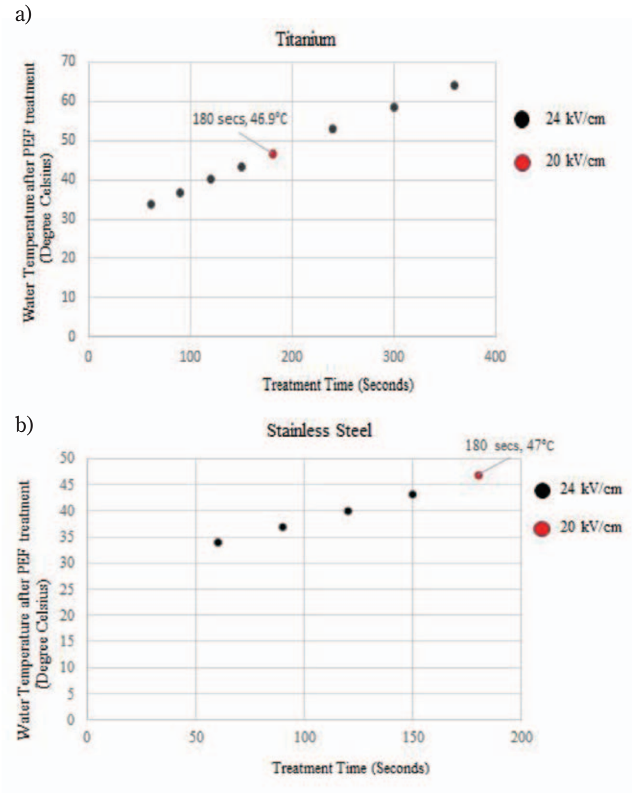

Fig. 5 Linear relationship between treatment time and temperature for a) Titanium electrodes b) Stainless Steel electrodes.

The red dot in Fig. 4 depicts reduced field from 24 kV/cm to 20 kV/cm but the curves are maintained still linear for both titanium and stainless steel electrodes. It is hence understood that rather than electric field, the treatment time played a primary role on both bacterial inactivation and temperature rise in the case of water. Though treatment time was increased, the associated pulsewidth was made short and constant throughout the experiment. This aided in getting complete absence of bacteria in water at less temperature. Despite adopting same methodology for both titanium and stainless steel electrodes, the highly reactive nature of titanium became advantageous for PEF in providing better results than stainless steel.

Based on the results obtained, PEF can thus effectively treat water using titanium electrodes by overcoming the drawbacks of existing water treatment systems. Though the use of titanium has been recommended for it's excellent electrochemical stability in PEF treatments, only very few studies have used the metal on liquid food products [18, 27]. Hence titanium needs further growth in usage and wide applications are required in different food products. From the observations, the potential of titanium is realized as a highly suitable material for PEF applications in water.

4. Conclusion

This research work has shown positive results on the use of titanium over stainless steel. (i) Titanium has shown to be more effective than stainless steel on inactivation of *E.coli* and *Coliform* bacteria, which was possible at a much lesser time of 60 seconds while stainless steel required 120 seconds for reaction (ii) Complete ABSENCE of bacteria was possible at 33.9C using titanium which was found to be lower than temperature requirement of 40.1℃ in stainless steel under the same experimental conditions. It is also understood that treatment time had a primary impact on temperature increase than electric field. Future research is based on pH analysis, filtering of impurities and inactivation of other bacterial loads in water to make PEF a complete technology for water decontamination. Hence the combination of anticorrosive and highly reactive titanium along with ultra short PEF application provides effective bacterial inactivation on drinking water at a very short treatment time with low temperature requirement.

Acknowledgements

The authors are thankful to Dr. Zarir Hector Sholapurwala, Chairman and Managing Director, M/s Zeonics Systech Defence R and D Lab, Bangalore, India for the experimental support.

For microbiological support, the authors are thankful to Mr. Bharath Mandyaramashetty, microbiologist, Eurofins Analytical Services, Bangalore, India.

References

1) J. E. Dunn, J. S. Pearlman; Methods and apparatus for extending the shelf life of fluid food products. U. S. Patent 4,695,472 (1987).
2) M. Sack, J. Sigler, C. Eing, L. Stuckenbrock, R. Stangle, A. Wolf, G. Muller; Operation of an electroporation device for grape mash, IEEE Trans. Plasma Sci., **38**, 1928–1934 (2010).
3) Y. Oka, Y. Muramoto, N. Shimizu, S. Ichihara; Sterilization of liquid by high electric field pulse. IEEE Conf. Electr. Insul. Dielectr Phenom., VA, 466–469 (2009).
4) S. Tseng, T. Wu, M. Wu; Bipolar narrow-pulse generator with energy-recovery feature for liquid-food sterilization. *IEEE Trans. Ind. Electron.*, **55**, 123–132 (2008).
5) B. L. Qin, Q. Zhang, G. B. V. Canovas, B. G. Swanson, P. D. Pedrow; Inactivation of microorganisms by pulsed elec-

tric fields of different voltage waveforms. IEEE Trans. on Dielectr. Electr. Insul., **1**, 1047 – 1057 (1994).

6) Q. Zhang, G. V. B. Canovas, B. G. Swanson; Engineering aspects of pulsed electric field pasteurisation. J Food Eng., **25**, 261 – 281 (1995).

7) C. Schultheiss, H. Bluhm, H. G. Mayer, M. Kern, T. Michelberger, G. Witte; Processing of sugar beets with pulsed–electric fields. IEEE Trans. Plasma Sci., **30**, 1547–1551 (2002).

8) A. H. El–Hag, O. R. Gonzalez, S. H. Jayaram, M. W. Griffiths; A performance study of a multilevel electrode treatment chamber for food processing. IEEE Trans. Ind. Appl., **49**, 1091–1097 (2013).

9) S. Qin, I. V. Timoshkin, M. Maclean, M. P. Wilson, M. J. Given, T. Wang, J. G. Anderson, S. J. Macgregor; Pulsed electric field treatment of saccharomyces cerevisiae using different waveforms. IEEE Trans. Dielectr. Electr. Insul., **22**, 1841 – 1848 (2015).

10) A. Gad, S. H. Jayaram; Effect of electric pulse parameters on releasing metallic particles from stainless steel electrodes during pef processing of milk. IEEE Trans. Ind. Appl., **50**, 1402–1409 (2014).

11) A. Gad, S. H. Jayaram, M. Pritzker; Performance of electrode materials during food processing by pulsed electric fields. IEEE Trans. Plasma Sci., **42**, 3161 – 3166 (2014).

12) L. C. Covington, R. W. Scutz; "Corrosion Resistant of titanium" TIMET Corporation, 1982.

13) B. S. Mitchell; "An introduction to materials engineering and science" John Wiley & Sons, New Jersey, 2004, p. 893 – 894.

14) Erick Butler, Yung–Tse Hung, Ruth Yu–Li Yeh, Mohammed Suleiman Al Ahmad; Electrocoagulation in Wastewater Treatment. Water, **3**, 495–525 (2011).

15) G. Patermarakis, E. Fountoukidis; Disinfection of water by electrochemical treatment. Water Research, **24**, 1491 – 1496 (1990).

16) K. Alexander**;** Electrochemical water disinfection: A short review. Platinum metals Rev., **52**, 177–185 (2008)

17) K. A. Athmaselvi, C. Kumar, P. Poojitha; Influence of temperature, voltage gradient and electrode on ascorbic acid degradation kinetics during ohmic heating of tropical fruit pulp. J. Food Meas. Charact., **11**, 144 – 155 (2016).

18) R. A. H. Timmermans, H. C. Mastwijk, L. B. J. M. Berendsen, A. L. Nederhoff, A. M. Matser, M. A. J. S. Van Boekel, M. N. Nierop Groot; Moderate intensity pulsed electric fields as alternative mild preservation technology for fruit juice. Int. J. Food microbiol., **298,** 63 – 73 (2019).

19) Kozisek, Frantisek; Health Risk from Drinking Demineralized Water. Rolling Revision of the WHO Guidelines for Drinking Water Quality, 148 – 163 (2004).

20) T. F. Wu, S. Y. Tseng, M. W. Wu, Y. M. Chen; Narrow pulsed voltage generator for liquid food sterilization. IEEE Appl. Power Electronics Conf. and Exp., Dallas, TX, 1354 – 1360 (2006).

21) K. H. Schoenbach, R. P. Joshi, R. H. Stark; Bacterial decontamination of liquids with pulsed electric fields. IEEE Trans. Dielectr. Electr. Insul., **7**, 637–645 (2000).

22) G. B. V. Canovas, M. M. G. Nieto, U. R. Pothakamury, B. G. Swanson; "Preservation of foods with pulsed electric fields" Academic Press, San Diego, CA, 1999, p. 7.

23) João P. S. Cabral; Review: Water microbiology. bacterial pathogens and water. Int. J. Environ. Res. Public Health, **7**, 3657–3703 (2010).

24) S. Qin, I. V. Timoshkin, M. Maclean, M. P. Wilson, S. J. MacGregor, M. J. Given, J. G. Anderson, Tao Wang; Pulsed electric field treatment of microalgae: Inactivation tendencies and energy consumption. IEEE Trans. Plasma Sci., **42**, 3191 – 3196 (2014).

25) R. E. Aly, R. P. Joshi, R. H. Stark, K. H. Schoenbach, S. J. Beebe; The effect of multiple, microsecond electrical pulses on bacteria. IEEE International Conference on Plasma Science, NV, USA, **2**, 1114–1117 (2001).

26) R. Bobinaitė, G. Pataro, N. Lamanauskas, S. Šatkauskas, P. Viškelis, G. Ferrari; Application of pulsed electric field in the production of juice and extraction of bioactive compounds from blueberry fruits and their by–products. J Food Sci Technol., **52**, 5898–5905 (2015).

27) H. Q. Zhang, G. V. B Cánovas, V. M. B. Balasubramaniam, C. P. Dunne, D. F. Farkas, J. T. C. Yuan; "Nonthermal processing technologies for food" IFT Press, USA, 2011, p. 211.