Utilization Certificate

Innovation Project 2015-16
SHC - 305

Project Title Industrial Waste Utilization For Microbial Fuel Production

Audited Financial Statement under Innovation Project scheme

College: Shivaji College

Project Investigators: Dr. Anita Kapur, Dr. Renu Baweja, Dr. Divya Mohanty

Grant Sanctioned Rs	Rs. 5,00,000/- (Rupees Five Lacs Only)		
	Grant Received	**Grant Utilized**	**Unspent Grant**
Equipments/Consumables	2,25,000/-	2,74,044/-	(49,044/-)
Travel	55,000/-	8,404/-	46,596/-
Stipend	1,20,000/-	1,20,000/-	NIL
Honorarium	25,000/-	25,000/-	NIL
Stationery	20,000/-	15,550/-	4,450/-
Contingency	55,000/-	53,006/-	1,994/-
Total	**5,00,000/-**	**4,96,004/-**	**3,996/-**
Total amount utilized	Rs. 4,96,004/- (Rupees four Lacs ninety six thousand four only)		
Amount remaining Rs. (In figures and words)	Rs. 3,996/- (Rupees Three thousand nine hundred ninety six only)		

Certified that out of **Rs. 5,00,000/- (Rupees Five Lacs Only)** sanctioned to Innovation Project Code **SHC-305, Rs. 4,96,004/- (Rupees four Lacs ninety six thousand four only)** has been utilized during the period of the project. The remaining amount **Rs. 3,996/- (Rupees Three thousand nine hundred ninety six only)** and is being returned back to the University.

Note : Over expenditure under the head "Equipment/Consumables" has been met from unspent balance in "Travel" with prior approval from the Innovation Desk.

Anita Kapur
1st Project Investigator

Renu.
2nd Project Investigator

Divya Mohanty
3rd Project Investigator

Principal

VIPUL DHAWAN & ASSOCIATES
CA
M.No. 518146
NEW DELHI
Chartered Accountants

04.11.16

University of Delhi

RC/2015/9435 31 August, 2015

The Principal,
Shivaji College
Ring Road, Raja Garden,
New Delhi-27

Subject: - **Innovation Projects 2015-16**

Dear Principal,

The University of Delhi is pleased to announce the third round of the undergraduate research initiative in colleges, Innovation Projects 2015-16. You will be glad to know that the following project submitted by your college has been selected for award

Project Code: SHC 305
Project Title: Agro-Industrial Waste Utilization For Biofuel Production

The distribution of grant under different budget heads as below:

Sr. No.	Budget Head	Amount
1.	Equipment/Consumables	Rs 2,25,000/-
2.	Stipends	Rs. 1,20,000/- (1000x10x12)
3.	Travel	Rs 55,000/-
4.	Honorarium	Rs 25,000/-
5.	Stationery/Printing	Rs 20,000/
6.	Contingency	Rs 55,000/-
	Total	Rs 500,000/-
Rs 5 lakhs (Rupees five lakhs only)		
Amount to be released in first phase by Finance Branch- Rs 3,50,000/		

Budget head No. 1 and half of the remaining grant will be released as the first instalment. The second and final instalment will be released after submission of half-yearly report (by 15 February 2016), satisfactory review and recommendation of release of the second instalment.
Please refer to the detailed guidelines for implementation of the project. Any queries may be addressed to- innovationprojects1516@gmail.com.

With best wishes,

Yours sincerely,

Prof. Malashri Lal

University of Delhi

INNOVATION PROJECT 2015-2016

SHC-305

(SHIVAJI COLLEGE)

Industrial Waste Utilization for Microbial Fuel Production

Industrial waste utilization for Microbial Fuel Production

Final Project Report

Delhi University Innovation Project SHC-305, 2015-16

Shivaji College

University of Delhi

Raja Garden

New Delhi-110027

Submitted by

Project Investigators:

Dr. Anita Kapur

Dr. Renu Baweja

Dr. Divya Mohanty

Mentor:

Dr. Nandita Moitra

Final Report

UNVERSITY OF DELHI
INNOVATION PROJECTS 2015-16
FINAL REPORT

1. PROJECT CODE : **SHC-305**

2. PROJECT TITLE : **Industrial Waste Utilization for Microbial Fuel Production**

3. NAME OF COLLEGE/INSTITUTION : **Shivaji College**

4. PRINCIPAL INVESTIGATORS (NAME, DEPARTMENT, EMAIL, PHONE NO.)

Name	**Department**	**Email ID**	**Mobile**
Dr. Anita Kapur	Botany	anitakapur52@gmail.com	91-9899068060
Dr. Renu Baweja	Biochemistry	renubaweja11@yahoo.com	91- 9818192670
Dr. Divya Mahanty	Botany	divyamohanty13@gmail.com	91-9810848968

5. MENTOR: Dr Nandita Moitra, Sr. Scientist, Research Development Planning & Co-ordination Cell, Water Lab, DPCC

6. STUDENTS INVOLVED IN THE PROJECT (NAME, DEPARTMENT, EMAIL ID AND PHONE NUMBER)

S.No.	**Name**	**Course and Year**	**Email ID and Mobile No.**
1.	Tarushi	B.Sc.(H) Biochemistry II nd Year	Tarushi1006@gmail.com, 91-9639559510
2.	Twinkle	B.Sc.(H) Biochemistry II nd Year	twinklrsharma3434@gmail.com, 91-9278927830
3.	Priyanka	B.Sc.(H) Biochemistry II nd Year	annanyasharma51@gmail.com, 91-8750065110
4.	Pratibha	B.Sc.(H) Biochemistry II nd Year	pratibhasodha123@gmail.com 91-9717428783
5.	Divyanka Chauhan	B.Sc.(H) Biochemistry IIIrd Year	divchauhan1996@gmail.com, 91-7042703003
6.	Anjali Nagar	B.Sc.(H) Biochemistry IIIrd Year	anjalinagar25@mail.com, 91-7838047667
7.	Puru	B. Tech Computer Science IVth Year	purusachan80@gmail.com 91-9990846995
8.	Drishti	B.Sc. (H) Zoology IIIrd Year	drishtygirdhar1994@gmail.com 91-9560139839
9.	Prachi	B.Sc. (H) Zoology IIIrd Year	prachipanwar560@gmail.com, 91-9871873736
10.	Ishender	B.Sc. (H) Zoology IIIrd Year	ishaankwatra@outlook.com 91-9541359816

University of Delhi

Certificate of Originality

This is to certify that the research work carried out and the final report submitted by the Project Investigators and the students of Innovation Project having Project code **SHC-305** and title **Industrial Waste Utilization for Microbial Fuel Production** of College/ Institute **Shivaji College** is original. Any plagiarism/academic dishonesty reported at any stage will be our responsibility.

Signatures of the all PIs

Abstract

Water pollution has always been a serious concern for the entire world, posing a threat on the health and survival of human beings, plants and animals both at present times and as well as for future. The industrial revolution and other anthropogenic activities are prime causes. As this problem of water pollution cannot be eradicated at once, it necessitates employment of various possible control measures to improve the conditions either by focusing on methods to reduce the waste or ensuring proper treatment of the generated waste. One of the upcoming ways to reduce the pollution caused by the industrial effluent is either to treat them with Microbial Fuel Cell (MFCs) or to use them for the production of MFCs so that the microbes present in effluent sample can be exploited to generate power. MFC or biological fuel cell is a bio-electrochemical system utilizing the natural metabolisms of the microbes. The microbes from effluent waste water can catalyze organic substrates by oxidation-reduction reactions and produce energy in the form of electricity or hydrogen. In the present research work, we have collected and characterize the wastewater effluent sample from mother dairy plant located at Patparganj, New Delhi. We determined its physical, chemical and microbiological parameters. After this, the effluent water sample was also employed to construct MFC. Under the experimental conditions we are successful in the construction of MFC with the maximum of 7.02 Volt.

Introduction

Industrialization and rapid urbanization have considerably increased the rate of water pollution. The environmental protection agencies have imposed more stringent regulatory prohibitions to protect the environment [1]. This has made wastewater treatment very expensive and difficult to handle. Moreover, the discharge of poor quality of effluent, results in pollution of water resources which poses a serious threats to human beings and aquatic organisms as they rely on these water resources for their survival. This problem is more severe and complex in developing countries where rapid increase in population and industrialization has raised the complexity of the effluent generated in various industries.

The food processing industries utilize water to meet their individual day-to-day needs. Dairy industry is one of the major food industries of India and contributes 35% of the total Asian milk [1]. Dairy industries require large quantity of water for various purposes including washing of cans, machinery and floor. Water is used in various stages of dairy operations. The quantity of liquid waste generated from manufacturing processes, utilities and service section. Water is used

for the processing in the ratio of 1:10 (water: milk) per litre of milk and there is approximately 0.2-1.0 L of waste water generated per L of processed milk [1].

Effluent from dairy industries is characterized by typical parameters that describe the nature of the waste and its potential impact on the environment. These parameters can be divided into physical, chemical and biological and include temperature, pH, oil and grease content, Total Suspended Solids (TSS), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). This wastewater when discharged without proper treatment, results in low Dissolved Oxygen (DO) and high Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). Moreover, the effluent from dairy industry is also rich in plethora of microorganisms. All these parameters of industrial effluents varies from industry to industry and even within industry, depending upon the various factors, such as size of operation and number of products manufactured, levels of processing, material conveyance, cleanup and housekeeping, and process water usage [2].

Dairy industry wastewater represents a complicated system containing different components. In composition, dairy wastewaters are neutral or slightly alkaline. This wastewater may rapidly become acidic due to the fermentation of milk sugar to lactic acid. It has also high content of fats, oil and grease which has negative impacts on wastewater treatment systems. Due to high organic load, wastewater from dairy effluent is characterized by high levels of chemical oxygen demand (COD), biochemical oxygen demand (BOD), oil and grease, nitrogen and phosphorus [3]. The Central Pollution Control Board (CPCB) has recommended the pH of the dairy industrial effluent to range between 6.5-8.5; a BOD of 100 mg/L (3 days at 27°C); suspended solids at a maximum concentration of 150 mg/L and oil and grease to be present at no more than 10 mg/L [4]. It is utmost important to characterize the industrial effluent to check their pollution potential. Once various physical, chemical and biological parameters are known, effective method of treatment can be designed/ used for the wastewater generated from these industries.

The effluent sample is treated before being discharged to the environment to reduce its deleterious effects on the environment. The final effluent released by such plants after the treatment can be used for irrigation and sludge itself becomes a good fertilizer [5]. Since past many years, methods have been introduced for effective treatment of waste water generated from dairy industry. One such method is the use of Microbial Fuel Cell (MFC) [6]. Microbial Fuel Cell is a Bioreactor which is capable of converting chemical energy stored in chemical

compounds in a biomass to electrical energy with the aid of micro-organisms. MFCs are used to produce electricity from renewable resources without the net carbon-di-oxide emission. During this process, along with the production of electrical power, the wastewater in the anode chamber is used as a substrate for treatment. Although carbon dioxide is produced during the oxidation process, its dissemination with this technology is low; because the carbon dioxide from renewable substrates originates from photosynthesis in the atmosphere and therefore the amount of carbon dioxide produced is natural [7]. Figure: 1 shows a schematic diagram of MFC with its various parts. It consists of an anode chamber where fuel/substrate is being oxidized to generate electrons and protons. These electrons are absorbed by anode and transfer to cathode through an external circuit [6]. The transfer of electrons must be matched by the number of protons moving between the electrodes in order to preserve electroneutrality. As shown in Figure: 1, the two chambers are connected via Proton Exchange Membrane (PEM). This is a salt-bridge through which protons enters into cathode chamber where they combine with oxygen to form water. Electric current is generated as microbes are present in anode chamber which is also an anaerobic chamber and hence no end-terminal acceptor or oxygen is present here [6].

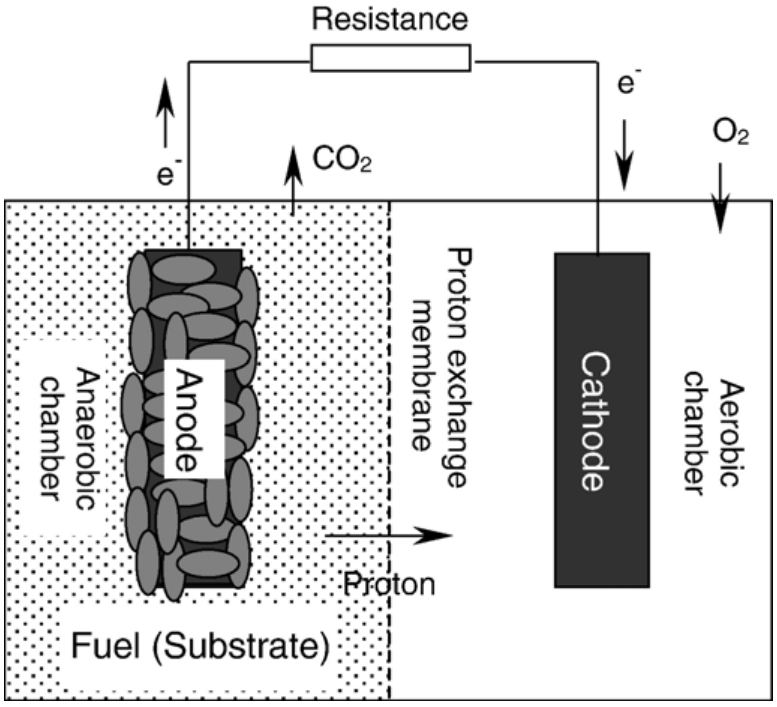


Figure: 1. Schematic diagram of a typical two chamber Microbial Fuel Cell [6]

Many microbes have been reported to be useful biocatalyst for the MFCs. Marine sediments; soil, fresh water sediments, wastewater and activated sludge are all rich sources of these types of

micro-organisms [6]. During the past few years, many different types of wastewater or effluent samples have been used for the production of MFCs. In one of the study, water from the Yamuna River was used to construct MFCs [8]. MFCs have been specifically used for the treatment of Municipal wastewater effluent also [6]. However, not much work has been carried out for the construction of MFCs using dairy effluent. In 2015, Patil et.al, construct MFC using lactobacillus species inoculated in dairy industry effluent sample [9]. But very less voltage (maximum of 0.471V) has been generated through this MFC.

Through our preliminary research work, we have constructed MFC using Mother Dairy Industrial effluent sample with an objective to provide a sustainable and efficient model which not only provides cleaner and renewable form of energy but also provides a method for cleaning the effluent discharge from dairy industries. Through the microbial analysis of effluent sample, we aim to analyze how the micro-organisms present in the effluent water could be used to produce such an efficient and green energy model. We desire such a simulation model in our experiment which not only gives good result in terms of potential difference across the cell but is also sustainable and doesn't degrade in a couple of days because of excessive bacterial interactions.

Research Problem/Hypothesis/Objectives

The current study is an attempt to monitor and analyze the quality of Dairy industry effluent released into the environment and employing this waste water for energy generation by a microbial fuel cell (MFC). Effluent sample (treated and untreated) has been collected from Mother Dairy Plant located at Patparganj, New Delhi, for the analysis of various physical and chemical parameters like BOD, COD, TSS, pH and temperature. We also aimed to analyse the sample for the presence of pesticides and heavy metal content. Following this, the effluent sample was analyzed for the presence of microbes and confirm their identity.

Further, the sample was employed for the generation of energy by construction and utilization of a microbial fuel cell which converts the chemical energy stored in the biodegradable substrate to electrical energy via microbial catalyzed redox reaction. The Microbial Fuel Cells (MFCs) provides an attractive option for the production of clean energy by the use of waste water generated from Dairy effluents with high organic content.

Following are the objectives of our research work:

1. Sampling, Preservation and Storage of the industrial waste sample
2. Measurement of the various physical parameters of the sample including colour, pH, Temperature, TDS, TSS, BOD, and COD.
3. Detection of the presence of organic and inorganic metal content in the effluent sample.
4. Detection of the presence of pesticides in the effluent sample.
5. Biochemical identification of the microbes present in waste water sample using commercially available test kits
6. Identification of microbes by 16S rRNA gene by PCR technique.
7. Generation of bioenergy from waste water sample using Microbial Fuel Cell.

Materials and Methods

Sample collection and storage

The sample was collected from Mother Dairy Plant (located at Patparganj, Delhi) and for our study we collected inlet or untreated effluent sample. For BOD, effluent sample was collected in BOD bottles and for metal analysis it was collected in glass bottles (treated with HNO_3). All the bottles/containers used for collection of sample were sterile. Special precautions were taken while collecting the sample for Biological parameters such as Total Plate Count. The outlet of the effluent sample was sterilized under the flame by the spirit lamp before collection of sample. Samples for analysis of metals and pesticides and for microbial count were stored at 4^oC after the collection. Temperature of the sample was measured at the site of sampling by dipping the standard thermometer inside the water for few minutes. For pH, BOD, COD and TSS, samples were processed immediately after the collection.

Physio-chemico analysis of effluent sample

The Untreated effluent sample was processed immediately post-collection to ensure that storage had no effect on its physical and chemical parameters. The pH, temperature, TSS, BOD and COD were determined or estimated using pH meter, thermometer and titrimetric methods, respectively as per the Central Pollution Control Board (CPCB) standard [10].

Analysis of Heavy metals and Pesticides

For the analysis of both heavy metals and pesticides, the effluent sample was given to FICCI Research Analysis Centre, Dwarka.

Analysis of Microbiological Parameters

The effluent sample was also analyzed for Total Microbial Count (plate count). Media used in this study were prepared and sterilized as per manufacturer instructions. For the total plate count, Luria Bertani Agar (LB agar) plates were used. The media was weighed and prepared in a conical flask. 200 ml LB agar was poured in 500 ml conical flask and kept for autoclaving. Once autoclaved, it was allowed to cool down to 55^0C - 60^0C and then poured into petriplates. After pouring, plates were incubated at room temperature for 18-24 hours to check for any contamination during the preparation. In estimating total plate count, these plates were aseptically inoculated with serial dilutions (10^{-1} to 10^{-8}) of untreated effluent by spread plate technique. For each plate, 20 µl of diluted culture was poured in the center of the plate and then spread using a glass spreader. These plates were further incubated at 37^oC for 18-24 hours for the growth. The number of colonies on plates was counted after incubation and CFU/ml was calculated by using following formula: CFU/ml = Number of colonies X Dilution factor X 1000/20.

Isolation of different strains of bacteria

Colonies with different morphological characteristics were selected and marked on the master plate. Further, pure cultures were developed by streaking them on a separate LB agar plate. After streaking, each plate was incubated at 37 ^{0}C for 18-24 hrs for the growth. Once pure culture is obtained, all the plated were stored at 4 ^{0}C for future use.

Morphological and Biochemical Characterization

The isolated strains were identified by morphological and biochemical characterization according to Bergy's Mannual of Determinative Bacteriology [11]. Colony morphology of each isolated strain was studied by growing individually on an LB agar plate. We also performed gram staining for each isolated strain. Different Biochemical tests were carried out using HiMedia Biochemical test kits (KB010, KB013, KB004). The different tests includes: Indole, Voges Proskauer, Citrate utilization tests, catalase test, malonate utilization and carbohydrate

fermentation of various sugars as per the instructions of the manual of commercially available kits.

Growth Curve for Isolated Strains

The LB broth medium was prepared and distributed in test tubes (5ml in 20 ml capacity test tubes) and conical flasks (80 ml in 250 ml capacity conical flasks) respectively and sterilized by autoclaving at 121 °C for 15 min. After cooling, each test tube was inoculated with a colony from three different isolated strains and incubated at 37 ºC for 18-24 h. Next morning, the three different conical flasks carrying 80 ml media each was labelled as Type-I, Type II and Type-III for three different stains. 5% of the inoculum prepared overnight for each culture was added to these three different flasks and mixed well. From each flask, 1 ml of culture was taken out and store at 4 ^{0}C to be used as 0 hour reading. Then, the flasks containing the culture were incubated at 37 ºC, 200 rpm. After every hour, 1ml of culture was taken and its absorbance was calculated at 620 nm. Microbial growth was measured up to 8hrs and the plot of Absorbance (620 nm) versus Time (hrs) was plotted for all three isolated stains.

16S rRNA sequencing identification

DNA of the three types of strains was isolated from the raised pure cultures using HiPer® Bacterial Genomic DNA Extraction (Himedia HTBM008) as per the instruction manual and its quality was verified on 0.8% Agarose Gel. After that, the genomic DNA sample and of pure cultures of the three types of strains were sent for sequencing to Eurofins. PCR amplification of the 16S rDNA fragment of gene was done using 27F and 1492R primers. Forward and Reverse DNA sequencing reaction of PCR amplicon was carried out with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the database of NCBI genbank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4 [12].

Microbial Fuel Cell construction and Operation

The cathode and anode chambers of MFC were constructed using the cost effective plastic boxes of 2 L capacity each available in local market. They were sealed with the sealing clay after the

addition of required effluent sample in the anode chamber and distilled water in cathode chamber. In the anode, the anaerobic conditions are maintained by purging with nitrogen. Nafio^{R}117 membrane (sigma) was used as a proton exchanger for the transfer of hydrogen ions. The cathode and anode were made of carbon cloth which was surrounded in an aluminum mesh. The effluent sample was put in anode chamber which was stirred continuously using the magnetic stir bar and operated at room temperature. Glucose at a concentration of 3gram/liter was used as a substrate and methylene blue was used as a mediator (300 µM). The amount of electricity generated was measured in the form of voltage using a multimeter (Sanwa CD 770) at regular intervals. Figure: 2 shows the experimental set of MFC.

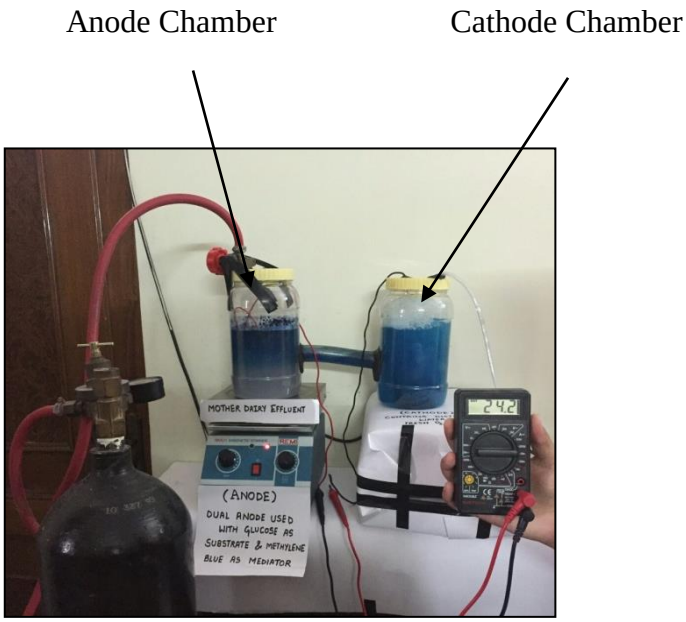


Figure: 2. Experimental setup of MFC.

Result and Discussion

Physio-chemico analysis

Water sample (treated and untreated) was collected from Mother Dairy Plant and analyzed for pH, Temperature, BOD, COD and TSS. The pH of the water sample for the treated and untreated samples were observed to be 7.5 and 6.5 and the temperature was 25 ºC and 37 ºC, respectively. The TSS was 280 mg/l for the untreated sample and 23.21 mg/ml for the treated sample. The value for BOD and COD was 115 mg/l and 312 mg/l respectively for the untreated sample. For the treated sample these values are comparatively low. The BOD was only 12.19 mg/dl and COD was 49.65 mg/dl [Figure: 3].

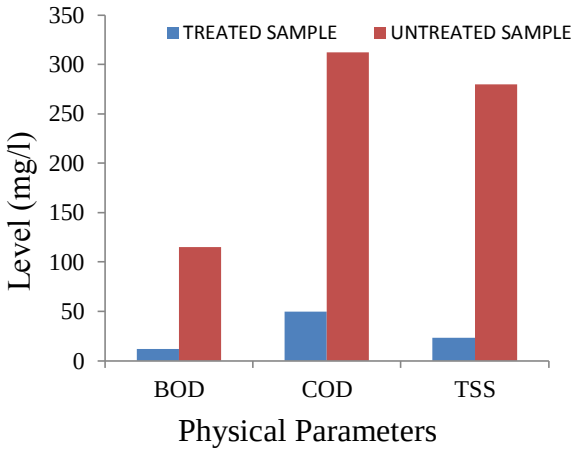


Figure: 3. Bar graph for physical parameters (treated and untreated)

As per the Delhi Pollution Control Committee (DPCC) Standard [9], the pH of the effluent sample should be in between 6.0-9.0 and the pH of the sample collected from Mother Dairy effluent was observed to be 6.5 and 7.5 respectively for the untreated and treated samples, suggesting that they are in the standard range. Moreover, it is close to 7.0 which is an optimum pH to support the growth of variety of microorganisms required for the production of MFC.

Biochemical Oxygen Demand (BOD) is an important parameter for the measurement of organic strength of waste water. The permissible limit for the BOD for dairy industrial effluent is 100 mg/l. It gives the measure of the amount of oxygen consumed by living organism (mainly bacteria) while utilizing organic matter in waste water [10]. The BOD value of our sample was observed to be 115 mg/l. This value indicates that the microbial load of our sample is relatively high compared to standard.

The Chemical Oxygen Demand (COD) value gives a measure of organic and in-organic pollutants which can be chemically oxidized. During the determination of COD, organic matter is converted to carbon dioxide and water regardless of biological assimilability [13]. The COD value for the effluent sample collected in the present work was found to be 312 mg/l as compared to the standard which is 250 mg/l. The COD values of effluent sample are usually 1.5 times BOD values [10]. But in our sample, it is comparatively high suggesting the presence of greater amount of oxidizing organic material in the effluent sample.

Total Suspended Solids (TSS) defines total solid particles which remain suspended in water in the form of colloids [14]. It is also one of the water quality parameter. The permissible limit for TSS for the dairy industrial effluent is 100 mg/l [10]. In the waste water sample collected by us,

its value was observed to be 280 mg/l which is approximately three times higher than the permissible limit suggesting the presence of large number of solid suspended particles and hence the pollutants. The more pollutants in water, the more will be the expected growth of microorganisms. This water therefore can be exploited for the generation of fuel by making Microbial Fuel Cell.

Analysis of Heavy metals and Pesticides

The untreated effluent sample was analyzed for both heavy metals and pesticides as per the specifications of FICCI Research Analysis Center (Test Method: FRAC/SOP/INST/242, 234, 300, 317 respectively). No detectable levels of Heavy metal and pesticides were observed in the effluent sample [Annexure I].

Analysis of Microbiological Parameters

The Total Plate Count of the effluent sample (treated and Untreated) was calculated as discussed in material and methods and observed to be 11 CFU/ml for the untreated sample and $2X10^6$ CFU/ml for the untreated sample [Table 1]. The total plate count for the treated sample was very low as compared to untreated sample suggesting the presence of sufficient number of microbes in an untreated sample as required for the production of Microbial Fuel Cell. Therefore, for further work we proceeded with the untreated sample as we require sample with good microbial load so that it can be exploited for the construction of Microbial Fuel Cell.

SAMPLE	**TOTAL PLATE COUNT/ml**
Mother Dairy (treated)	11
Mother Dairy (untreated)	$2X10^6$

Table: 1. CFU/ml of treated and untreated sample

Isolation of different strains of bacteria

We have observed three different types of colonies on the LB agar plate of untreated effluent sample. These three different microbial strains were marked and labelled as Type-I, Type-II and Type-III [Figure: 4].

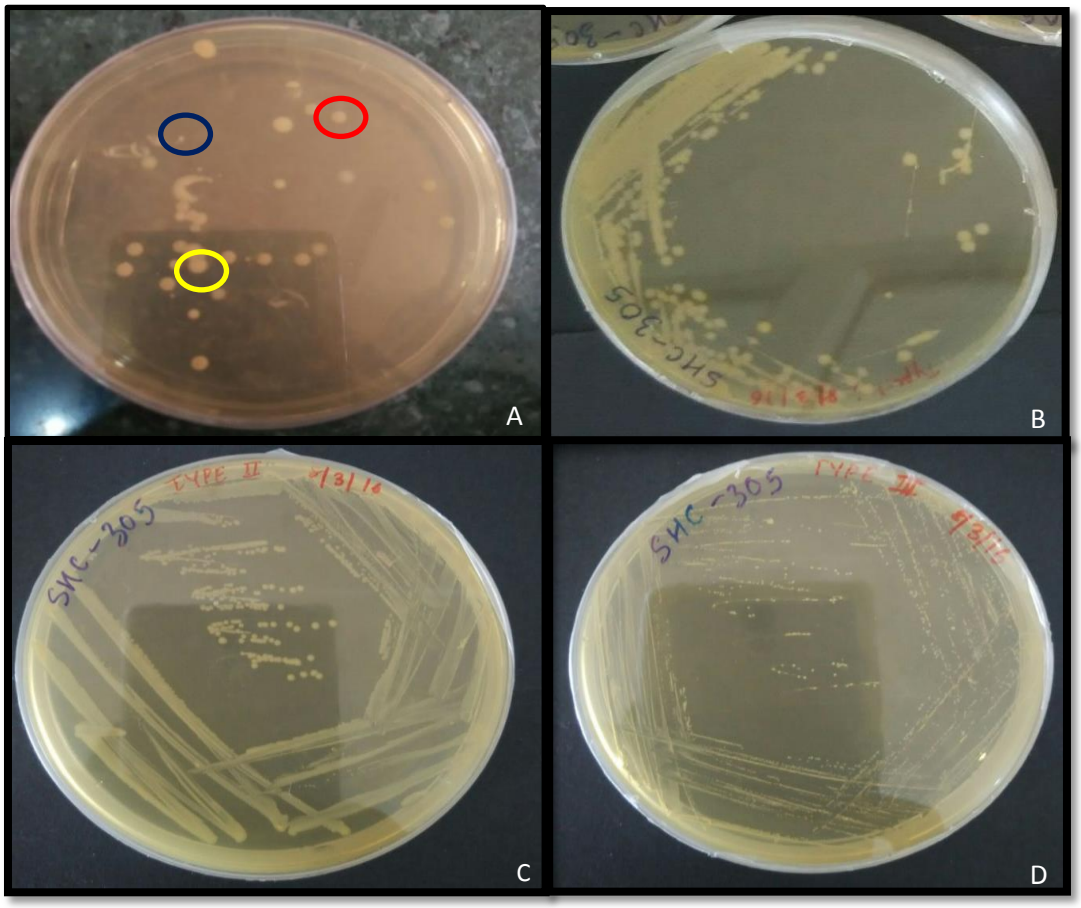


Figure: 4(A-D). Colonies observed after plating the effluent sample (Masterplate: A; Type I- red circle, Type II- yellow circle and Type III- blue circle). Streaked plates of Type I, II and III culture (B-D).

Growth Curve for Isolated Strains

The plot of absorbance versus time for three isolated strains shows sigmoidal curve with lag, log and stationary phase. Because of the time constrain, the growth curve was plotted for 8hrs. It was observed that both type-II and type-III strain enters into log phase after 1 hr of incubation compared to the type-I which remains in lag phase for approximately 3 hrs. Moreover, the log phase for type-II and type-III was also observed to be more compared to type-I. All the culture observed to enter into stationary phase after 8hrs of incubation at 37^0C, 200 rpm.

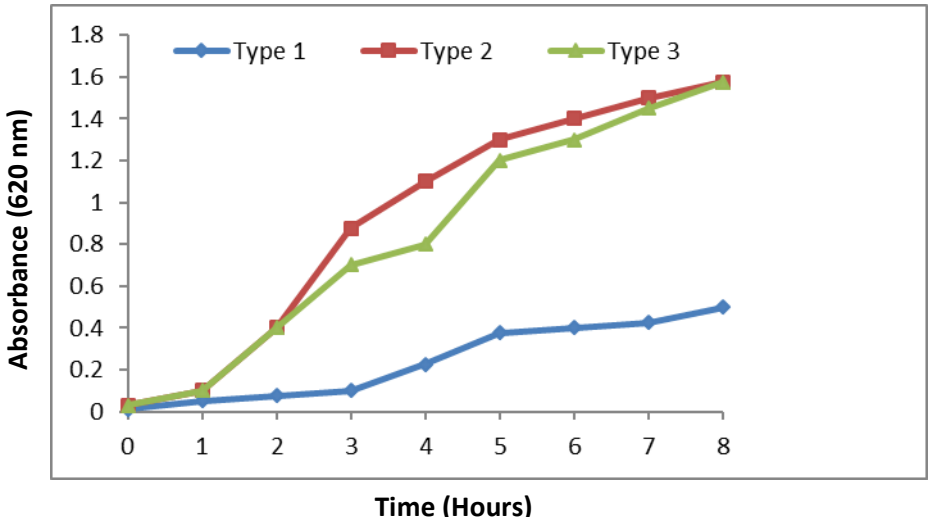


Figure: 5. Growth curve for the three types of strains.

Morphological and Biochemical Characterization

In order to identify the bacteria, it is very important to know its culture characteristics. Agar based media can be used to identify colony characteristics which includes its shape, size, elevation and margin as different types of bacterial species will produce different looking colonies on an agar plate [15]. The results of morphological features of different types of colonies along with their shape and gram staining characteristics are mentioned in the Table 2.

Type-I culture shows typical rod-shape bacteria. They are arranged in chains. Moreover Type-II was variable with respect to its staining characteristics as it shows both purple and red color of typical gram positive and gram negative bacteria. Moreover we were unable to identify its proper shape as it appears to be cocci or small rods. Type-III culture was identified to be gram positive cocci with most of the cells arranged in bunch.

Culture Identity	**Characteristics**		
	Morphological Features of the Colony	**Shape of Bacteria**	**Gram Staining**
Type-I	White, Large, Round, Opaque with irregular margin	Bacilli	Positive
Type-II	Yellow, Round, Entire Opaque	Bacilli/Cocci	Positive/Negative
Type-III	White , Punctiform, Round	Cocci	Positive

Table: 2. Morphological features of three different types of colonies

The bacterial identification kits are standardized, colorimetric test systems based on substrate utilization and other biochemical tests. These tests are specific for identification of specific bacterial species based on the principle of pH change and substrate utilization that is exhibited by color change in the media when it is incubated for specific time period with the bacterial culture.

Initially, HiE. coli™ Identification Kit (Himedia KBM0001) was used but the result did not match with any of *E. coli* strain indicating that none of the three strains were *E. coli.*

Next on using the HiBacillus™ Identification Kit (Himedia KB013) which has a combination of 12 tests for identification, the following results were obtained [Table 3].

S. No.	Test	Type 1	Type 2	Type 3
1.	Malonate	-	-	-
2.	Voges Proskauer's	+	-	+
3.	Citrate	+	-	+
4.	ONPG	-	+	_
5.	Nitrate Reduction	+	+	+
6.	Catalase	+	-	-
7.	Arginine	+	-	+
8.	Sucrose	+	-	+
9.	Mannitol	-	+	-
10.	Glucose	+	+	+
11.	Arabinose	-	+	-
12.	Trehalose	+	+	-

Table 3: Reactions observed using HiBacillus™ Identification Kit by incubating the three types of strains.

According to this, Strain Type 1 was identified as *Bacillus cereus* (gram positive bacteria). It also matched with the bacterial shape observed during Gram staining i.e. rod shaped. It could utilize Citrate, Glucose, Trehalose and displays a delayed reaction for utilization of Arginine, Sucrose. Also it gave a positive reaction for Voges Proskauer's test which detects acetoin production, Catalase and Nitrate reduction test. But it failed to utilize Malonate, Mannitol, Arabinose or detect β-galactosidase activity with ONPG test.

Thereafter, HiStaph^{TM} Identification Kit (Himedia KB004) was used which is also a combination of 12 tests (some are different than that for *Bacillus*). With its help Strain Type 3 was identified as *Staphylococcus epidermidis*. It also matched with the bacterial shape observed during Gram staining. i.e. spherical. It could utilize Arginine, Sucrose, Lactose, Maltose but failed to utilize Mannitol, Arabinose, Raffinose, Trehalose. The presence of acetoin production, Alkaline Phosphatase and Urease was detected but β-galactosidase activity was absent.

Strain 2 could not be identified with any of the three kits used indicating that it was neither of the three strains- *E. coli, Bacillus* or *Staphylococcus.*

16SrRNA sequencing identification

One of the genetic markers that have been employed for bacterial identification, taxonomy and phylogeny is the most common housekeeping gene sequence i.e. 16S rRNA [16]. It is due to the following reasons:

(i) Presence in almost all bacteria, often existing as a multigene family,

(ii) Function of the 16S rRNA gene over time has not changed,

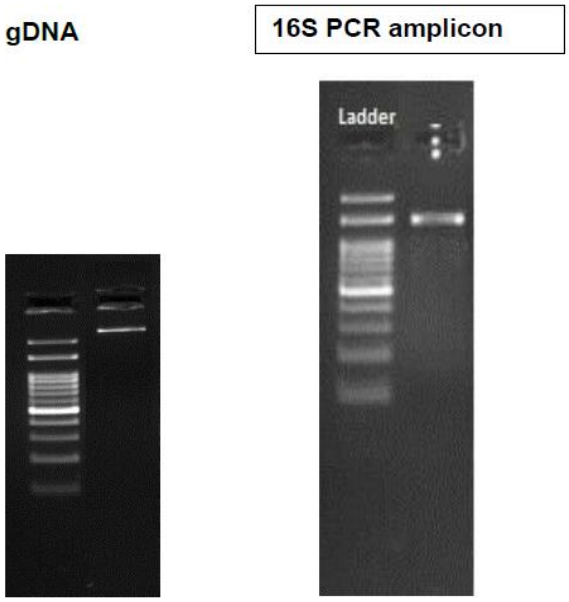
(iii) 16S rRNA gene (1,500 bp) is large enough for bioinformatics purposes [17]

Thus, 16S rRNA gene sequencing is the most attractive way of genus and species identification that may not fit any standard biochemical profile. Upon total genomic DNA extraction and gel verification, a single band of high-molecular weight DNA was observed. After PCR amplification, a single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose gel.

- ***Type I***

Sample labelled as TYPE_I was found to be ***Bacillus cereus*** strain based on nucleotide homology and phylogenetic analysis.

Agarose Gel Electrophoresis of gDNA and PCR amplification



Bioinformatics: Fasta sequence, BLAST Analysis and Phylogenetic Tree Construction

>TYPE1_consensus sequence

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GCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACT
GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGC
CGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCA
CCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTA
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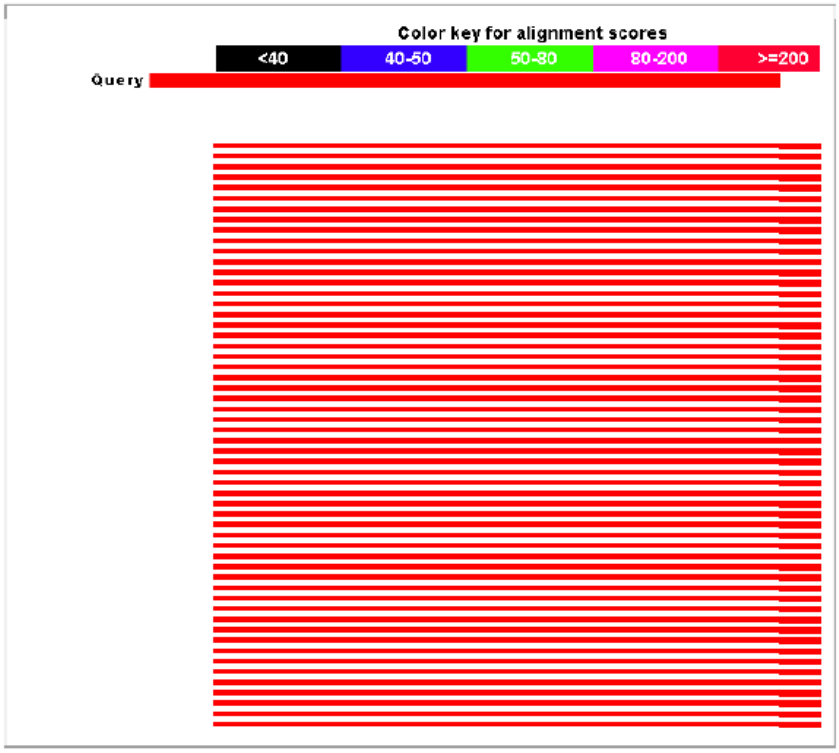
Alignment view using NCBI GenBank

Distribution of 100 Blast Hits on the Query Sequence



Sequences producing significant alignments:

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Bacillus cereus strain DF30 16S ribosomal RNA gene, partial sequence	2230	2230	100%	0	100%	KU976421.1
Bacillus cereus strain DF29 16S ribosomal RNA gene, partial sequence	2230	2230	100%	0	100%	KU976420.1
Bacillus cereus strain DP26 16S ribosomal RNA gene, partial sequence	2230	2230	100%	0	100%	KU976419.1
Bacillus cereus strain DP23 16S ribosomal RNA gene, partial sequence	2230	2230	100%	0	100%	KU976418.1
Bacillus cereus strain HTP03 16S ribosomal RNA gene, partial sequence	2230	2230	100%	0	100%	KX024730.1
Bacillus cereus strain X2 16S ribosomal RNA gene, partial sequence	2230	2230	100%	0	100%	KU991849.1
Bacillus cereus strain S5 16S ribosomal RNA gene, partial sequence	2230	2230	100%	0	100%	KU927490.1
Bacillus cereus strain KTNC0124 16S ribosomal RNA gene, partial sequence	2230	2230	100%	0	100%	KT968367.1

Phylogenetic Tree

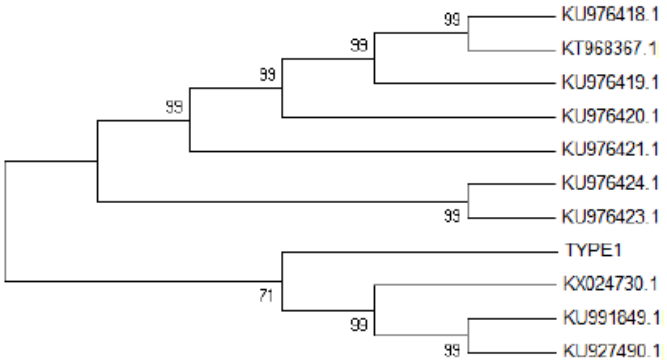


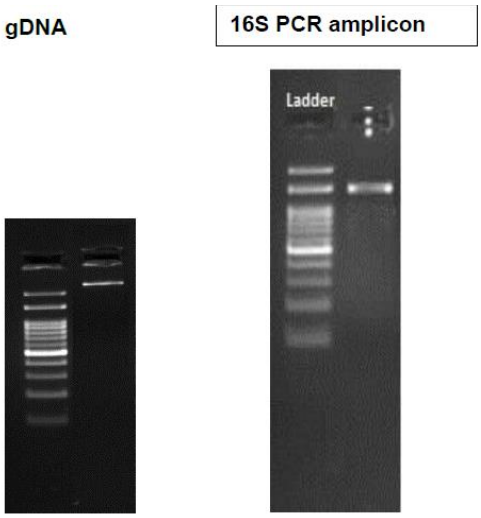
***Figure*. Evolutionary relationships of 11 taxa**

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.00072411 is shown. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test (500 replicates is shown next to the branches [2, 3]. The evolutionary distances were computed using the Kimura 2-parameter method [4] and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 1382 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [5].

- ***Type II***

Sample labelled as TYPE_II was found to be ***Citrobacter freundii*** strain based on nucleotide homology and phylogenetic analysis.

Agarose Gel Electrophoresis of gDNA and PCR amplification



Bioinformatics: Fasta sequence, BLAST Analysis and Phylogenetic Tree Construction

>TYPE II_consensus sequence

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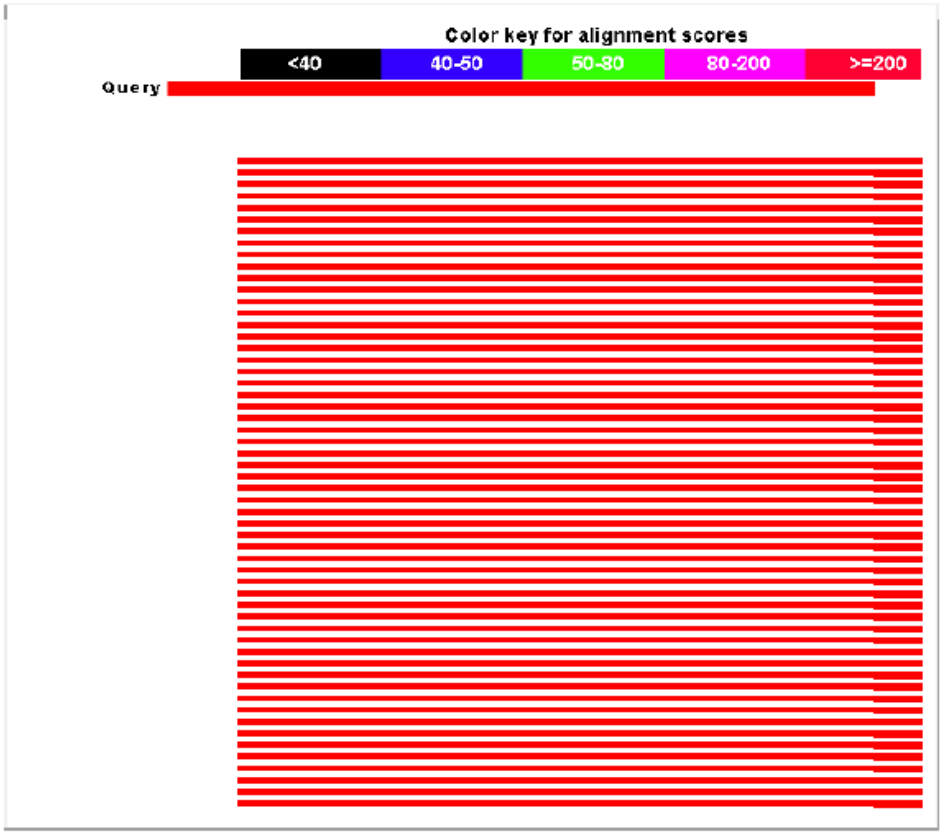
Alignment view using NCBI GenBank

Distribution of 100 Blast Hits on the Query Sequence



Sequences producing significant alignments:

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Citrobacter braakii strain Hb-7 16S ribosomal RNA gene, partial sequence	2361	2361	100%	0	99%	KC139411.1
Citrobacter murliniae strain BAC041 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0	99%	KU161313.1
Citrobacter freundii strain N52 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0	99%	KT719221.1
Citrobacter freundii strain C11 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0	99%	KF145194.1
Citrobacter gillenii strain MN51 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0	99%	KM289144.1
Citrobacter braakii strain DSM 17596 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0	99%	NR_117750.1
Citrobacter freundii strain sch23 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0	99%	JX294897.1
Citrobacter freundii strain sch40 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0	99%	JX294881.1
Citrobacter freundii 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0	99%	HQ170626.1

Phylogenetic Tree

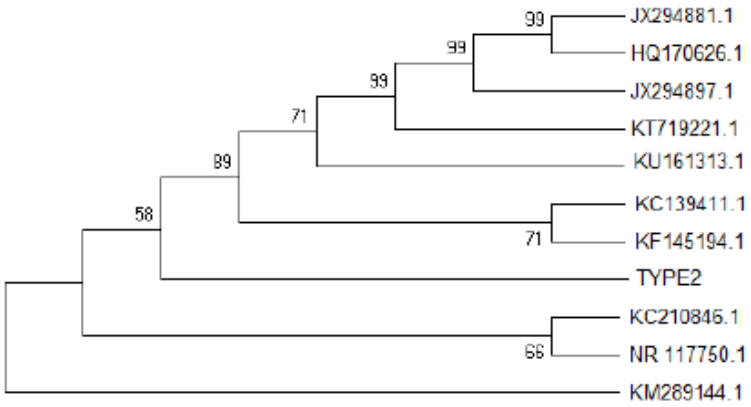


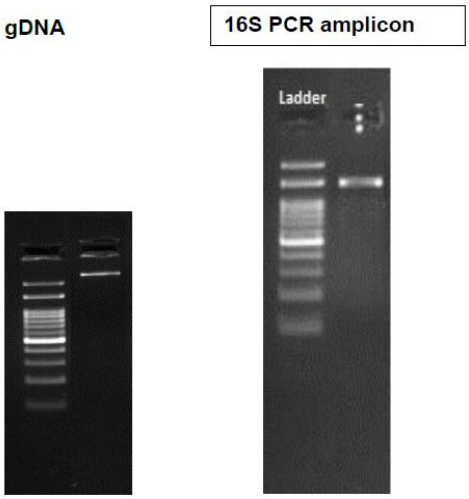
***Figure*. Evolutionary relationships of 11 taxa**

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.01283354 is shown. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test (500 replicates is shown next to the branches [2, 3]. The evolutionary distances were computed using the Kimura 2-parameter method [4] and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 1368 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [5].

- ***Type III***

Sample labelled as TYPE_3 was found to be ***Staphylococcus epidermidis*** strain based on nucleotide homology and phylogenetic analysis.

Agarose Gel Electrophoresis of gDNA and PCR amplification



Bioinformatics: BLAST Analysis and Phylogenetic Tree Construction

>TYPE III_consensus seq

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GATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTT
AGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAAC
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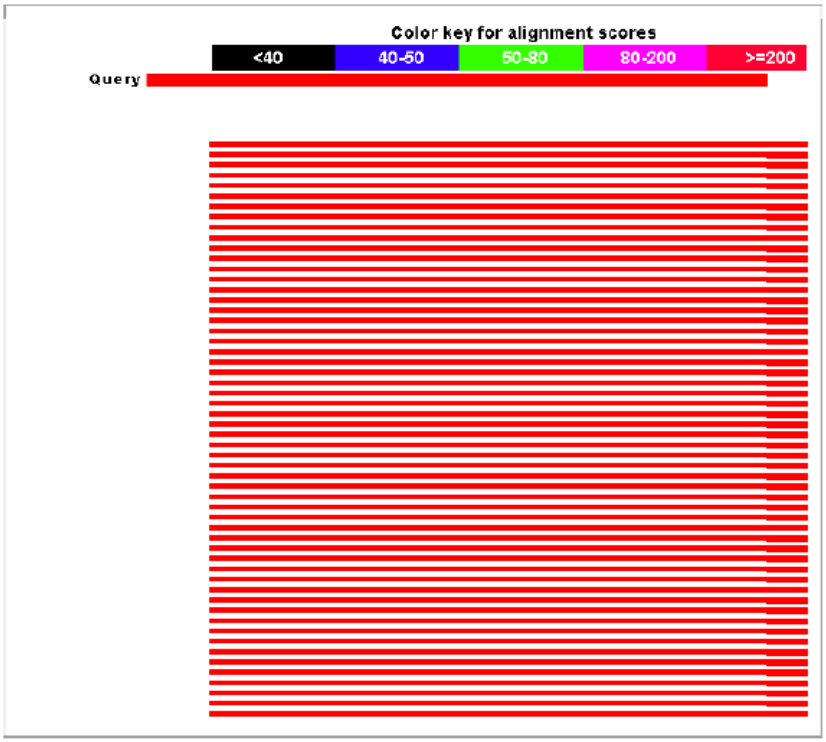
Alignment view using NCBI GenBank

Distribution of 100 Blast Hits on the Query Sequence



Sequences producing significant alignments:

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Staphylococcus epidermidis strain IARI-CDK 18 16S ribosomal RNA gene, partial sequence	2405	2405	100%	0	99%	KT441071.1
Staphylococcus epidermidis strain SE4.8 16S ribosomal RNA gene, partial sequence	2405	2405	100%	0	99%	KM877507.1
Staphylococcus epidermidis strain SE4.7 16S ribosomal RNA gene, partial sequence	2405	2405	100%	0	99%	KM877506.1
Staphylococcus epidermidis strain SE4.6 16S ribosomal RNA gene, partial sequence	2405	2405	100%	0	99%	KM877505.1
Staphylococcus epidermidis strain SE2.9 16S ribosomal RNA gene, partial sequence	2405	2405	100%	0	99%	KM877504.1
Staphylococcus capitis subsp. capitis strain MER_TA_69.2 16S ribosomal RNA gene, partial sequence	2405	2405	100%	0	99%	KT719474.1
Staphylococcus epidermidis strain TWSL_19 16S ribosomal RNA gene, partial sequence	2405	2405	100%	0	99%	KT184899.1
Staphylococcus epidermidis strain KS3H17 16S ribosomal RNA gene, partial sequence	2405	2405	100%	0	99%	KJ571204.1
Staphylococcus epidermidis strain 23.1 16S ribosomal RNA gene, partial sequence	2405	2405	100%	0	99%	KM052316.1

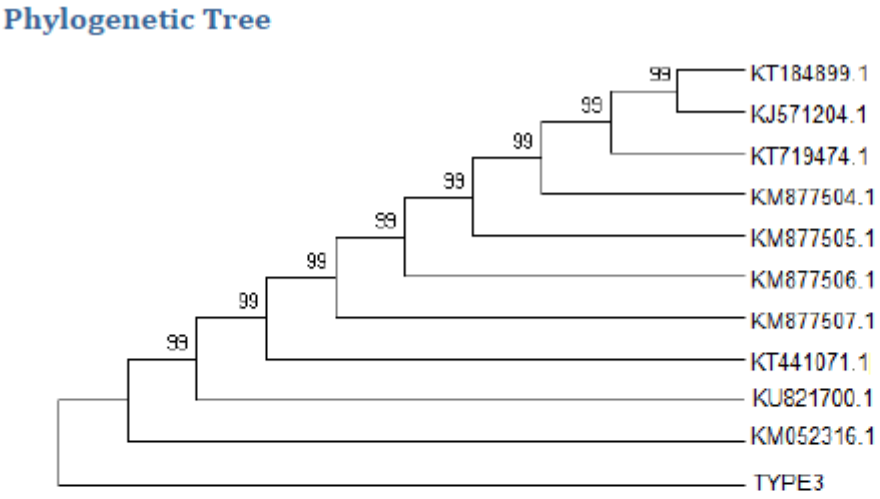

***Figure*. Evolutionary relationships of 11 taxa**

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.00076220 is shown. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test (500 replicates is shown next to the branches [2, 3]. The evolutionary distances were computed using the Kimura 2-parameter method [4] and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 1313 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [5].

Microbial Fuel Cell Construction and Operation

The working of MFC and hence the generation of electricity depends on the reactions carried out between microbes and organic substrates present in anode chamber [14]. The voltage output was recorded and plotted against time at regular intervals. Table 4 shows the readings of the voltage output at an interval of 2 hrs. These reading were the plotted against time. Figure: 6 shows the graph of Voltage (V) versus Time (hrs).

The graph between voltage and time shows that the initial voltage generated in MFC was 2.24 Volt. The voltage was recorded for 4 days after every 2 hrs. The peak was observed at the end of the third day with the voltage of 7.02 Volt. After that there is slight but continuous decline in voltage (Table 4).

S. No.	Time (Hours)	Voltage (V)	S. No.	Time (Hours)	Voltage (V)
1.	0	2.24	26.	50	6.68
2.	2	2.46	27.	52	6.79
3.	4	2.52	28.	54	6.63
4.	6	2.68	29.	56	6.52
5.	8	3.22	30.	58	6.43
6.	10	3.46	31.	60	6.41
7.	12	3.59	32.	62	6.38
8.	14	3.76	33.	64	6.32
9.	16	3.92	34.	66	6.44
10.	18	4.23	35.	68	6.52
11.	20	4.46	36.	70	6.64
12.	22	4.84	37.	72	6.83
13.	24	4.92	38.	74	7.02
14.	26	5.03	39.	76	6.99
15.	28	5.12	40.	78	6.96
16.	30	5.32	41.	80	6.89
17.	32	5.56	42.	82	6.82
18.	34	5.79	43.	84	6.76
19.	36	5.84	44.	86	6.71
20.	38	5.98	45.	88	6.65
21.	40	6.04	46.	90	6.62
22.	42	6.23	47.	92	6.6
23.	44	6.25	48.	94	5.9
24.	46	6.31	49.	96	5.8
25.	48	6.42			

Table: 4. Voltage generated up to 96 hours.

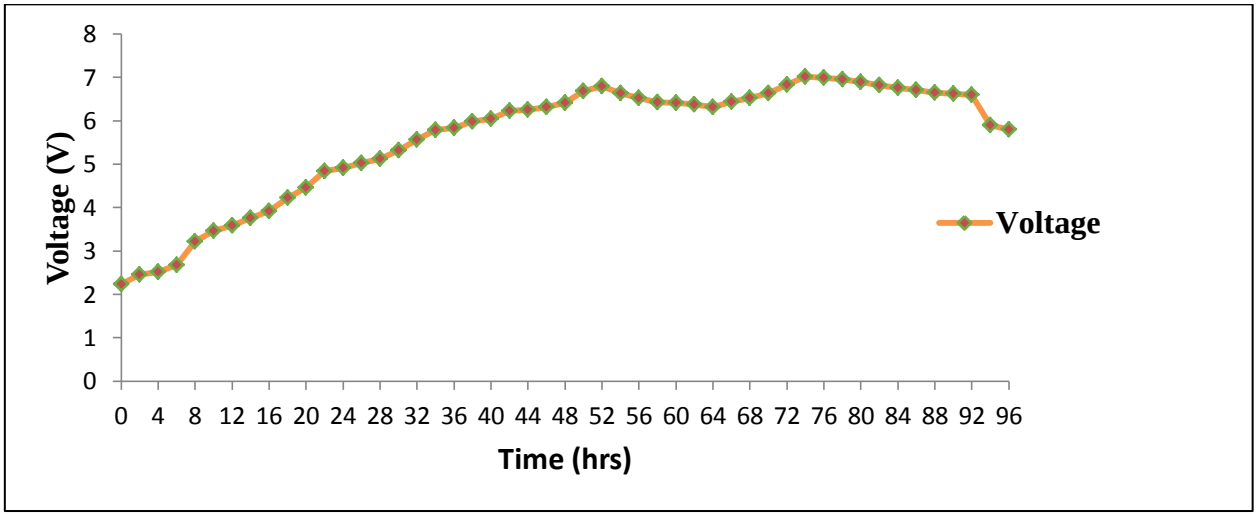

Figure: 6. Graph of Voltage versus Time.

The voltage generated by MFC constructed in the present work using dairy effluent is very high. One of the reasons for this is the presence of sufficient substrate in the anode chamber and the addition of methylene blue as a mediator. The mediators function as an oxidizing agent for respiratory proteins in the outer membrane of the micro-organisms and subsequently transfer the electrons obtained at anode [18]. Therefore, these mediators shuttle between the bacteria and anode transferring the electrons and hence increase the output. It has been reported previously that methylene blue at a concentration of 300µM act as very efficient mediator for MFC [18]. We have also used methylene blue at a concentration of 300 µM as a mediator and added it in the anode chamber. This result in high voltage output of MFC prepared in our study with the dairy wastewater effluent sample. Though, there are many reports where mediator-less MFCs have been constructed [8, 14, 19] from wastewater samples, but in our sample we have not observed the presence of various metal ions and other components which can serve as a mediator. Therefore, we decided to construct MFC in the presence of mediator for the efficient voltage output.

Innovations shown by the project

We have constructed Microbial Fuel Cell from the effluent sample from dairy industry. Though, there are many reports where MFCs are constructed using effluent samples from different industries [6, 8, 9], Very few reports are there where effluent from dairy industry has been employed for the construction of MFC. In one of the study [9], MFC was constructed using lactobacillus species inoculated in dairy industry effluent sample. But very less voltage (maximum of 0.471V) has been generated through this MFC. In another study [7], the dairy industry wastewater was used to construct MFC but again the maximum voltage generated was only 0.865 V was generated. Hence it is for the first time that microbial population of dairy industrial effluent sample was exploited for the construction of MFC with a very high voltage of 7.02 Volts.

Conclusion and Future direction

Through our preliminary research work, we have shown that wastewater from dairy industry can be used for the generation of sustainable energy using MFC. The power generation through MFC is affected by many factors including the type of bacteria or population of microbes present in an innoculum, the type of substrate and its concentration, ionic strength, pH,

temperature, materials used for electrodes & Proton Exchange Membrane and other reactor configuration [6]. We have used conventional method for the construction of MFC. Future optimizing studies can be attempted to improvise the power generation through MFC. Though Microbial Fuel Technology is still in its early stage of development but shows great promise as a new method for the sustainable electricity generation and wastewater treatment. Major issues to be solved for its practical application include its cost and power output.

References

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7. Mansoorian H.J, Mahvi A.H. Jafari A.J., Khanjani N. (2014) Evaluation of dairy industry wastewater treatment and simultaneous bioelectricity generation in a catalyst-less and mediator-less membrane microbial fuel cell. *Journal of Soudi Chemical Society*, 20: 80-100
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Publication/s from the work. (attach copies): Annexure II

Conference Presentation/s (attach copies): Annexure III

Patent/s and Technology Transfer (attach copies): None

Media Coverage (attach copies): None

Pictures related to the project: Figures 7-11 appended below.

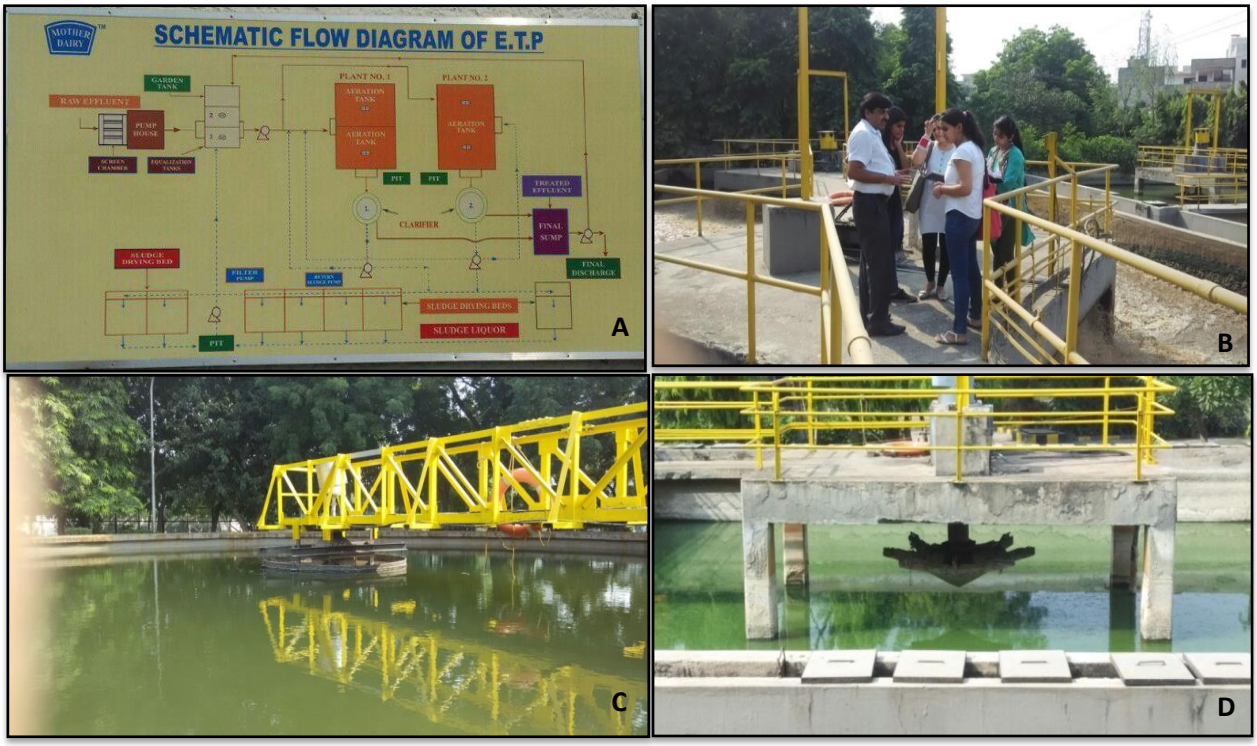

Figure: 7A-D. Visit to Mother Diary Plant located at Patparganj, New Delhi.
A. Schematic diagram of Effluent Treatment Plant; B-D. Treatment Plant.

Figure: 8A-D. Sample Collection in the Mother Diary Plant with the help of Senior Scientists Delhi Pollution Control Committee and technical staff Mother Diary.

Figure. 9 A-F. Students working in the DPCC lab with the sample collected at the Mother Diary Plant (B).

Figure: 10 A-D. Students interacting with mentor (A, B) and giving presentations after literature survey (C, D).

Figure11A-C. Poster presentation in Daulat Ram College (A), Shivaji College (B) and Gargi College (C)

Annexure-I

FICCI RESEARCH & ANALYSIS CENTRE

PLOT 2A, SECTOR-8, DWARKA, NEW DELHI-110077 Tel: 91-11-45333500-510 Fax: 91-11-25360802

E-mail : info@fraclabs.org, Website : www.fraclabs.org

TEST REPORT

061553

Issued to:
Shivaji College
University of Delhi

Kind Attn: Ms. Renu Bajwa

Sample Description:
Water

Sampling Method: Not Applicable

Reg. No.: FRAC/W/160108020-A
Your ref. No.: Not specified
Letter Dt: Not specified

Date of Issue: 19.01.2016
Date of Sample Recd.: 08.01.2016
Date of Sampling: Not Applicable
Date of Sample Booking: 08.01.2016

Batch No.: Not specified
Mfd. Date: Not specified
Exp. Date: Not specified
Sample Quantity: 500ml

TEST RESULTS

S. No.	Parameters	Results	Specification	Test Method
1.	Lead, mg/ltr	Not Detected	--	FRAC/SOP/INST/242
2.	Copper, mg/ltr	Not Detected	--	
3.	Arsenic, mg/ltr	Not Detected	--	
4.	Mercury, mg/ltr	Not Detected	--	
5.	Methyl Mercury, mg/ltr	Not Detected	--	FRAC/SOP/INST/234
6.	Tin, mg/ltr	Not Detected	--	FRAC/SOP/INST/300
7.	Zinc, mg/ltr	Not Detected	--	
8.	**Organochlorine Group, mg/ltr**			
	Alpha HCH	Not Detected	--	FRAC/SOP/INST/317
	Beta HCH	Not Detected	--	
	Gamma HCH (Lindane)	Not Detected	--	
	Delta HCH	Not Detected	--	
	Heptachlor and Epoxide	Not Detected	--	
	Aldrin, Dieldrin	Not Detected	--	
	Chlordane (cis & trans)	Not Detected	--	
	Alpha Endosulphan	Not Detected	--	
	Beta Endosulphan	Not Detected	--	
	Endosulphan Sulphate	Not Detected	--	
	Endrin	Not Detected	--	
	Endrin Aldehyde	Not Detected	--	
	Endrin Ketone	Not Detected	--	
	o.p DDE	Not Detected	--	
	p.p DDE	Not Detected	--	
	o.p DDD	Not Detected	--	
	p.p DDD	Not Detected	--	
	o.p DDT	Not Detected	--	
	p.p DDT	Not Detected	--	
	Methoxychlor	Not Detected	--	

Authorised Signatory

S. K. MANOCHA
Asstt. Director-Technical
Ficci Research & Analysis Centre
Dwarka, New Delhi-110077

Note:
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TEST REPORT

061554

Reg. No.: FRAC/W/160108020-A

9.	**Organophosphorus Group, mg/ltr**			
	Dichlorvos	Not Detected	--	FRAC/SOP/INST/317
	Monocrotophos	Not Detected	--	
	Phorate, Phorate sulphoxides, Phorate sulphones	Not Detected	--	
	Diazinon	Not Detected	--	
	Methyl parathion	Not Detected	--	
	Parathion	Not Detected	--	
	Fenitrothion	Not Detected	--	
	Malathion	Not Detected	--	
	Maloxan	Not Detected	--	
	Fenthion	Not Detected	--	
	Chlorpyrifos	Not Detected	--	
	Ethion and analogue	Not Detected	--	
	Phosalone	Not Detected	--	
	Phosphamidon	Not Detected	--	
	Chlofennvinphos and it's isomers	Not Detected	--	
	Phenthoate	Not Detected	--	
	Pirimphos methyl	Not Detected	--	
	Alachlor	Not Detected	--	
	Quinolphos	Not Detected	--	
	Butachlor	Not Detected	--	
10.	**Herbicides Group, mg/ltr**			
	2,4-D	Not Detected	--	FRAC/SOP/INST/317
	MCPA	Not Detected	--	
	Atrazine	Not Detected	--	
	Simazine	Not Detected	--	
	Hexaconazole	Not Detected	--	
	Isoproturan	Not Detected	--	
11.	**Pyrethroids, mg/ltr**			
	Permethrin	Not Detected	--	FRAC/SOP/INST/317
	Cypermethrin	Not Detected	--	
	Deltamethrin	Not Detected	--	
	Fenvalerate	Not Detected	--	
	Lambda Cyhalothrin	Not Detected	--	
12.	**Carbamates, mg/ltr**			
	Carbaryl	Not Detected	--	FRAC/SOP/INST/317
	Caarbofuran	Not Detected	--	

Detection Limit: Lead-0.008mg/l, Copper, Arsenic-0.01mg/l, Mercury-0.0005mg/l, Methyl mercury-0.1mg/, Tin, Zinc-0.05mg/l, Organochlorine, Organophosphorous, Herbicides, Pyrithroides-0.01mg/l, 2,4-D, Isoproturan-0.05mg/l

Remark: Customer asked for above tested parameters.

D.O.R.: 08.01.2016
D.O.C.: 19.01.2016

Authorised Signatory
S. K. MANOCHA
Asstt. Director-Technical
FICCI Research & Analysis Centre
Dwarka, New Delhi-110077

Note:
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Annexure-II

Publication/s from the work

1. Baweja, R., Kapur, A. (2016). Dairy effluent: a source for the production of bio-energy. *Journal of Energy Research and Environmental Technology*, 3, 88-90.

2. Baweja, R., Kapur, A., Mohanty, D., Girdhar, D., Panwar, P., Ishender, Tarushi, Twinkle, Priyanka, Pratibha, Anjali, Divyanka. (2016). Bio-electricity production from food industry effluent. In: Conference Proceedings of National Seminar on Water and Air Quality in Urban Eco-system on March 22, 2016 organized by Eco-Club, Shivaji College, University of Delhi, New Delhi – 110027, India. (ISBN No. 978-93-5267-493-0 *in Press*).

Journal of Energy Research and Environmental Technology (JERET)
p-ISSN: 2394-1561; e-ISSN: 2394-157X; Volume 3, Issue 2; April-June, 2016; pp. 88-90

http://www.krishisanskriti.org/Publication.html

Dairy Effluent: A Source for the Production of Bio-energy

Renu Baweja* and Anita Kapur#

**Department of Biochemistry, Shivaji College*
#Department of Botany, Shivaji College
*E-mail: *renubaweja11@yahoo.com*

Abstract—*The current global warming crisis can be mitigated by using renewable bio-energy. Production of Microbial Fuel is one such example. MFC is used to convert energy stored in chemical bonds in organic compounds to electric energy through the reactions catalyzed by microorganisms. This application of MFC has generated considerable interests among researchers in recent years. MFC can also be used to treat industrial wastewater. Through our research work, we attempt to make microbial fuel cell from the mother dairy effluent sample by exploiting the reaction catalyzed by the microbial population present in the sample. The cathode and anode of MFC were prepared using activated carbon cloth surrounded in an aluminum mesh. Glucose was used as a substrate and methylene blue was used as a mediator. The production of electricity was measured using a multimeter at regular intervals. Minimum of 2.24 Voltage and maximum of 7.02 Voltage was obtained during experimental conditions in the laboratory.*

Keywords*: MFCs, Bio-energy, Effluent*

1. INTRODUCTION

Water is an utmost requirement of human beings. Without water, life is not possible. Out of the total ground water available to humankind, only 0.29-0.49% is available in the form of drinking water. This necessitates the treatment and recycling of water [6]. It is not only a universal and precious solvent but also one of the abundantly available resources in ecosystem which can be utilized by human beings for their day-to-day activities. Since past few years, due to the uncontrolled population growth and intensive agricultural activities, ground and surface water has been exploited on a large scale. These activities finally lead to the major issues related to the public health like poor water quality, its safety and conservation [6].

Microbial Fuel Cell is a Bioreactor which is capable of converting chemical energy stored in chemical compounds in a biomass to electrical energy with the aid of micro-organisms. MFCs are used to produce electricity from renewable resources without the net carbon-di-oxide emission. Fig. 1 shows a schematic diagram of MFC with its various parts. It consists of an anode chamber where fuel/substrate is being oxidized to generate electrons and protons. These electrons are absorbed by anode and transfer to cathode through an external circuit [6]. The transfer of electrons must be matched by the number of protons moving between the electrodes in order to preserve electroneutrality. As shown in Fig. 1, the two chambers are connected via Proton Exchange Membrane (PEM). This is a salt bridge through which protons enters into cathode chamber where they combine with oxygen to form water. Electric current is generated as microbes are present in anode chamber which is also an anaerobic chamber and hence no end-terminal acceptor or oxygen is present here [6]

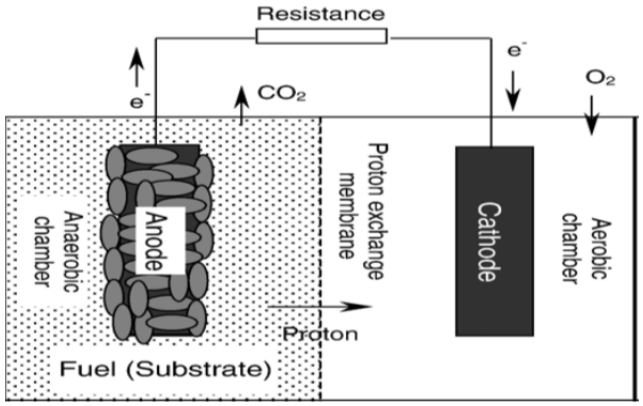

Fig. 1: Schematic diagram of a typical two chamber Microbial Fuel Cell [6]

Many microbes have been reported to be useful biocatalyst for the MFCs. Marine sediments; soil, fresh water sediments, wastewater and activated sludge are all rich sources of these types of micro-organisms [6]. During the past few years, many different types of wastewater or effluent samples have been used for the production of MFCs. In one of the study, water from the Yamuna river was used to construct MFCs [1]. MFCs have been specifically used for the treatment of Municipal wastewater effluent also [6]. However, not much work has been carried out for the construction of MFCs using dairy effluent. In 2015, Rahul et.al, construct MFC using lactobacillus species inoculated in dairy industry effluent

sample [5]. But very less voltage has been generated through this MFC. Through our research work, we have constructed MFC using Mother Dairy Industrial effluent sample collected from Mother Dairy Plant located in Delhi. The two chambers MFC was made using Glucose as a substrate and Methylene blue as a mediator.

2. METHODOLOGY

2.1 Sample collection and storage

The sample was collected from Mother Dairy Plant (located at Patparganj, Delhi) and for our study we collected inlet or untreated effluent sample in order to get good microbial population for MFC production. For MFC, the effluent sample was collected in a sterile plastic container and stored at room temperature.

2.2 Microbial Fuel Cell construction and Operation

The cathode and anode chambers of MFC were constructed using the cost effective plastic boxes of 2Liter capacity each available in local market. They were sealed with the sealing clay after the addition of required effluent sample in the anode chamber and distilled water in cathode chamber. In the anode, the anaerobic conditions are maintained by purging with nitrogen.Nafio^{R}117 membrane (sigma) was used as a proton exchanger for the transfer of hydrogen ions. The cathode and anode were made of carbon cloth which was surrounded in an aluminium mesh. The effluent sample was put in anode chamber which was stirred continuously using the magnetic stir bar and operated at room temperature. Glucose at a concentration of 3gram/liter was used as a substrate and methylene blue was used as amediator (300µM). The amount of electricity generated was measured in the form of voltage using a multimeter (Sanwa CD 770) at regular intervals. Fig. 2 shows the experimental set of MFC.

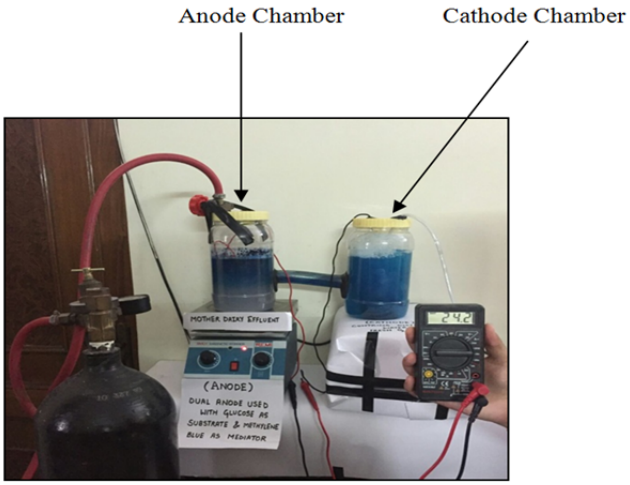

Fig. 2: Experimental setup of MFC

3. RESULT AND DISCUSSION

The working of MFC and hence the generation of electricity depends on the reactions carried out between microbes and organic substrates present in anode chamber [4]. The voltage output was recorded and plottedagainst time at regular intervals. Fig. 3 shows the graph of Voltage (V) versus Time (hrs).

The graph between voltage and time shows that the initial voltage generated in MFC was 2.24 Volt. The voltage was recorded for 4 days after every 2 hrs. The peak was observed at the end of the third day with the voltage of 7.02 volt. After that there is slight but continuous decline in voltage.

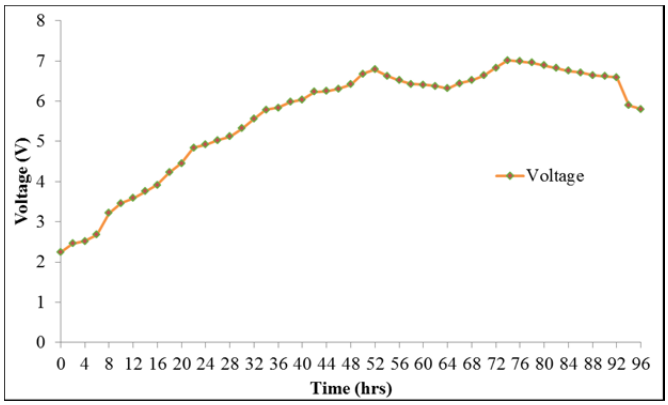

Fig. 3: Graph of Voltage versus Time

The voltage generated by MFC constructed in the present work using dairy effluent is very high. One of the reasons for this is the presence of sufficient substrate in the anode chamber and the addition of methylene blue as a mediator. The mediators function as an oxidizing agent for respiratory proteins in the outer membrane of the micro-organisms and subsequently transfer the electrons obtained at anode [2]. Therefore, these mediators shuttle between the bacteria and anode transferring the electrons and hence increase the output. It has been reported previously that methylene blue at a concentration of 300µM act as very efficient mediator for MFC [2]. We have also used methylene blue at a concentration of 300µM as a mediator and added it in the anode chamber. This result in high voltage output of MFC prepared in our study with the dairy wastewater effluent sample. Though, there are many reports where mediator-less MFCs have been constructed [1,3,4] from wastewater samples, but in our sample we have not observed the presence of various metal ions and other components which can serve as a mediator (result not shown). Therefore, we decided to construct MFC in the presence of mediator for the efficient voltage output.

4. CONCLUSION

Through our preliminary research work, we have shown that wastewater from dairy industry can be used for the generation of sustainable energy using MFC. The power generation through MFC is affected by many factors including the type of bacteria or population of microbes present in an innoculum, the type of substrate and its concentration, ionic strength, pH, temperature, materials used for electrodes & PEM and other reactor configuration [6]. We have used conventional method for the construction of MFC. Future optimizing studies can be attempted to improvise the power generation through MFC. Though Microbial Fuel Technology is still in its early stage of development but shows great promise as a new method for the sustainable electricity generation and wastewater treatment. Major issues to be solved for its practical application include its cost and power output.

5. ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support provided by University of Delhi under DU-Innovation Project SHC-305. We are also thankful to Puru Sachan, the student under this project for his assistance in carrying out the experimental work and our Principal Mam for the infrastructural support.

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[3] Gil GC, Chang IS, Kim BH, Kim M, Jang JY, Park HS, et al. Operational parameters affecting the performance of a mediatorless microbial fuel cell. *BiosensBioelectron* 2003;18:327–34.

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Annexure-III

Conference Presentation/s

1. **Poster presentation** in an International Conference on Public Health: Issues, Challenges, Opportunities, Prevention, Awareness on 15th and 16th January 2016 held at Daulat Ram College, University of Delhi
2. **Poster presentation** in the National Seminar on Water and Air Quality in Urban Eco-system on 22nd March, 2016 organized by Eco-Club, Shivaji College, University of Delhi
3. **Oral presentation** in an International Conference on Public Health: Issues, Challenges, Opportunities, Prevention, Awareness (Public Health: 2016) on 21st May 2016 organized by "Krishi Sanskriti" and held at Jawaharlal Nehru University, New Delhi.
4. **Poster presentation** in National Conference on Combating Industrial Pollution for Sustainable Environment- A fusion of Industrial and Science Efforts (CIPSE-2016) on 22nd - 23rd September, 2016 organized by Gargi College, University of Delhi

Characterization of Wastewater Effluent From Dairy Industry For Energy Generation

Dr. Renu Baweja[1], Dr. Anita Kapur[2], Ms. Divyanka Chauhan[3], Ms Anjali Nagar[3],

Email ID: renubaweja11@yahoo.com

[1]Assistant Professor, Department of Biochemistry, Shivaji College, University of Delhi
[2]Associate Professor, Department of Botany, Shivaji College, University of Delhi
[3]Students of B.Sc. (H) Biochemistry IV Semester, Shivaji College, University of Delhi

ABSTRACT

The wastewater from the dairy industries is highly putrescible. It possesses serious health problems due to the presence of various hazardous substances. It is one of the most polluting industries, not only in terms of volume of effluent generated, but in terms of characteristics as well. It generates 0.2-10 litres of effluent per year with an average value of 2.5 L/L of milk processed. Since dairy effluents decompose readily and pollute the environment, these industries are using waste water treatment methods before discharging this effluent.

The characteristics of a dairy effluent contain Temperature, Color, pH, DO, BOD, COD, Dissolved solids, suspended solids, chlorides, sulphate, oil & grease. The effluent sample could be a very good source of microbes as well. Depending upon the microbial composition we can use them for the generation of energy/fuel by making the Microbial Fuel Cell (MFC).

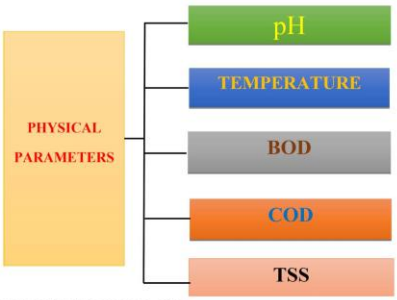
Through this study we aim to characterize the untreated and treated waste water effluent sample from Mother Dairy industry. We have determined the microbial content of both treated and untreated samples. The result of various characteristics including Biological Oxygen Demand, Chemical Oxygen Demand, Total Suspended Particles and Total plate count are reported in this study.

OBJECTIVES

- To investigate the various physical parameters of wastewater from dairy industry.
- To study the Total Plate Count of wastewater.

INTRODUCTION

Among the major industries in India, dairy is one of the industries producing wastewater rich in organic matter. It generates about 0.2–10 liters of effluent per liter of milk processed [1]. In general, liquid waste in dairy industry presents the following characteristics: high organic content, high oils and fats content, high level of nitrogen and phosphorous, dissolved sugar, nutrients, and so forth [2]. This waste water is also a very good source of microbes. This microbial population can be exploited for various applications. One of the important applications is the usage of bacterial population for the production of microbial fuel cell (MFC). MFC is an electrochemical system that drives a current by using bacteria. In this regard, it is also important to know the various physical and biological parameters of the wastewater to determine the quality of water and to determine the total microbial count and type of microbes in waste water.



METHODOLOGY

We have collected both treated and untreated waste water sample from mother dairy plant and determined the various physical parameters including temperature, pH, Total Suspended Solids (TSS), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) values. The samples for measuring various physical parameters were given to DPCC (Delhi Pollution Control Committee). We have also determined the total plate count of the waste water sample. For this, effluent sample collected for determination of microbial content was given to FICCI Analysis Centre, Dwarka, New Delhi.

Sample collecion from mother dairy plant

Woking at Delhi Pollution Control Committee (DPCC) Lab

RESULTS

Physical Parameters	Treated Sample	Untreated Sample
pH	7.5	6.5
Temperature	25°C	37°C
BOD	12.19 mg/l	115 mg/l
COD	49.65 mg/l	312 mg/l
TSS	23.21 mg/l	280 mg/l

FUTURE DIRECTIONS

We have observed that treated effluent sample from Mother Diary Plant is highly pure with respect to its microbial content. The BOD and COD values of untreated samples are quite high suggesting the higher microbial load. The plate count of the treated sample was 11 cfu/ml. Since the microbial content of the treated sample was too less for it to be used for the production of microbial fuel cell, we have also collected the unreated sample to determine its plate count and finally the microbial content.. Once we find the microbial population we will exploit this sample further for the production of microbial fuel cell.

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ACKNOWLEDGEMENT

The authors are thankful to our Menor Dr Nandita Moitra, Sr. Scientist, Research Development Planning & Co-ordination Cell, Water Lab, DPCC, University of Delhi for funding under Innovation Projects for Colleges, **Project Code SHC-305** and also to the student research group associated with this project in collecting the effluent sample.

Poster Presented in Daulat Ram College

Bioelectricity Production From Food Industry Effluent

Dr. Renu Baweja[*], Dr. Anita Kapur[#], Ms. Divya Mohanty[#], Drishty Girdhar[$], Prachi Panwar[$], Ishender[$], Tarushi[*], Twinkle[*], Priyanka[*], Pratibha[*], Anjali[*], Divyanka[*]

[*]Department of Biochemistry, Shivaji College, University of Delhi
[#]Department of Botany, Shivaji College, University of Delhi
[$]Department of Zoology, Shivaji College, University of Delhi

ABSTRACT

Water pollution has always been a serious concern for the entire world and posing a threat on the health and survival of human beings, plants and animals both at present times and as well as for future. The industrialization and other anthropogenic activities are prime causes. As this problem of water pollution cannot be eradicated at once, it necessitates employment of various possible control measures to improve the conditions either by focusing on methods to reduce the waste or ensuring proper treatment of the generated waste. One of the upcoming ways to reduce the pollution caused by the industrial effluent is either to treat them with Microbial Fuel Cell (MFCs) or to use them for the production of MFCs so that the microbes present in effluent sample can be exploited to generate power. MFC is a bio-electrochemical system utilizing the natural metabolisms of the microbes. The microbes from effluents can catalyze organic substrates by oxidation-reduction reactions and produce energy in the form of electricity or hydrogen. With the impending energy crises, this eco-friendly and multi-disciplinary approach is the need of the hour. Early 20th century witnessed the demonstrations of first MFCs while the turn of the 21st century saw its commercial application for wastewater treatment. Along with that, MFCs as a form of renewable energy can find applications as biosensors like for BOD sensing, for hydrogen production, as power supply to remote sensors and underwater devices.

INTRODUCTION

Water is essential to life. It needs not to be spelt out how exactly important it is. Yet water pollution is one of the most serious ecological threats we face today. As this problem cannot be eradicated at once, it necessitates employment of various possible control measures to improve the conditions either by focusing on methods to reduce the waste or ensuring proper treatment. MFCs are bioelectrical cell which can be generated using the power of microbes present in the effluent sample which otherwise leads to water pollution. Through our research work we tried to determine the various physical and biological parameters of Mother Dairy Food Industry so that it can be further used for the production of MFC.

OBJECTIVES

- Collection of Effluent Sample
- Determination of Physical Parameters
- Detection of Heavy Metals and Pesticides
- Characterization and Identification of Microbes
- Production of Microbial Fuel Cell

RESULTS

Total Suspended Solids (TSS)	280mg/l
Chemical Oxygen Demand (COD)	312mg/l
Biological Oxygen Demand (BOD)	115mg/l
Heavy metals and Pesticides	Not Detected
Total Plate Count	$2X10^6$ CFU/ml

FUTURE DIRECTIONS

- Characterization and Identification of Microbes
- Production of Microbial Fuel Cell

ACKNOWLEDGEMENT: Delhi University Innovation Project (SHC-305)

Poster Presented in Shivaji College

Identification of Microbes Isolated From Dairy Industrial Effluent

Dr. Renu Baweja*, Dr. Anita Kapur#, Ms. Divya Mohanty#, Drishty Girdhar$, Prachi Panwar$, Ishender$, Tarushi*, Twinkle*, Priyanka*, Pratibha*, Anjali*, Divyanka*

*Department of Biochemistry, Shivaji College, University of Delhi
#Department of Botany, Shivaji College, University of Delhi
$Department of Zoology, Shivaji College, University of Delhi

ABSTRACT

The waste water generated in a dairy industry consists of organic components that are highly putrescible. This waste water may pose serious health hazard due to the presence of solids and liquid effluents containing a spectrum of organic and inorganic chemicals. The effluent from dairy industry may also be the good source of variety of microorganisms. The population of microbes present in the effluent sample can be used for the production of Microbial Fuel Cell (MFC). A MFC is a bio-electrochemical system, which converts the chemical energy stored in the biodegradable substrate to electrical energy via microbial catalyzed redox reaction. Therefore, the Microbial Fuel Cell provides an attractive option for the production of clean energy by the use of waste water generated from dairy industrial effluent.
Through our research work, we attempt to analyse the quality of dairy industrial effluent specifically with respect to its microbial content. For this, the effluent water (treated and untreated) from mother dairy plant was collected and their total plate count was determined. Three different types of colonies were identified in an untreated sample under the laboratory conditions suggesting the presence of at least three different strains in the untreated effluent sample. Further, all the three isolated strains were characterized with respect to their colony morphology, gram staining and growth curve. We also tried to identify the strain of microbes using Biochemical Identification kits.
Through our preliminary work, we have identified three different strains as *Bacillus* gram positive, *cocci* gram negative/positive and *cocci* gram negative. One of the strain was identified as *Bacillus Cereus*. The three different strains will further be identified using 16S rRNA sequence analysis.

INTRODUCTION

Water is essential to life. Yet water pollution is one of the most serious ecological threats we face today. The waste water generated in a dairy industry consists of not only variety of organic and inorganic compounds but also a very good source of micro-organisms. The effluent from dairy industry may also be the good source of variety of microorganisms. The microbial population of waste water can be exploited for the production of Microbial Fuel Cell. A Microbial Fuel Cell is a bio-electrochemical system, which converts the chemical energy stored in the biodegradable substrate to electrical energy. This redox reaction is catalysed by microbes. MFCs can provide an answer to several of the problems which traditional wastewater treatment faces. They enable the recovery of energy out of the wastewater, while limiting both the energy input and the excess sludge production. Through this paper, we are presenting our work for the isolation and characterization of the waste water effluent sample of mother dairy industry specifically with respect to its microbial population.

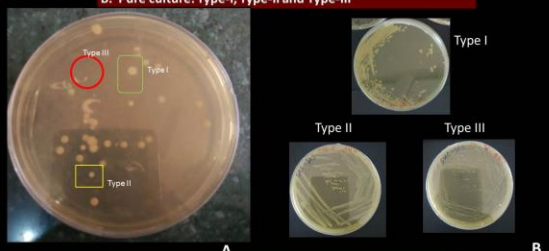
METHODOLOGY

- Untreated Effluent sample from Mother Dairy Industry plant was collected under aseptic conditions
- The sample was serially diluted in a Laminar hood and each dilution was plated on an LB agar plate. Each plate was then incubated at 37^{0}C overnight
- The CFU/ml of the culture was calculated.
- Three different types of colonies were also identified on a plate and they were further streaked on LB agar plate to get a pure culture. They were labelled as Type-I, Type-II and Type-III.
- Growth Curve analysis for these three different strains were carried out.
- The strains were further characterize by performing Gram Staining and various biochemical tests using HiIMViC™ Biochemical Identification Kit

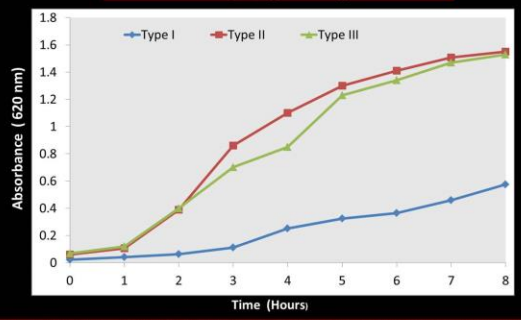
RESULT

Culture Identity	Colony Morphology	Shape of bacteria	Gram Staining
Type I	White, Large, Round, Opaque	Bacilli	Positive
Type II	Yellow, Round, Entire Opaque	Cocci	Positive/Negative
Type III	Yellow, Punctiform, Round	Cocci	Negative

A. The LB agar plate with three different types of colonies
B. Pure culture: Type-I, Type-II and Type-III



C. Growth curve of Type-I, Type-II and Type III cultures



D. Biochemical Identification using HiIMViC™ Biochemical Identification Kit: All the Tests mentioned were performed using kit for *Bacillus* species with three identified strains and Type I was identified as *Bacillus Cereus*

	TYPE 1	TYPE 2	TYPE 3
Malonate	-	-	-
Voges proskaeur's	+	-	-
Citrate	-	-	+
DNPG	-	+	+
Nitrate reduction	+	+	+
Catalase	-	-	-
Arginine	+	-	-
Sucrose	-	-	-
Mannitol	-	+	+
Glucose	+	+	+
Arabinose	-	+	+
Trehalose	+	+	+

FUTURE DIRECTIONS

- Identification of three different strains using 16srRNA sequence analysis.
- Production of microbial fuel cell using waste water sample from mother dairy plant

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