# Generation of green electricity from sludge using photo-stimulated bacterial consortium as a sustainable technology

Amal S. Othman[1], Nashwa A. Ahmed[1*], Mona S. Elneklawi[1], Mansour M. Hassan[2] and Mahmoud Abd El-Mongy[2]

## Abstract

Microbial fuel cell (MFC) is a bio-electrical energy generator that uses respiring microbes to transform organic matter present in sludge into electrical energy. The primary goal of this work was to introduce a new approach to the green electricity generation technology. In this context a total of 6 bacterial isolates were recovered from sludge samples collected from El-Sheikh Zayed water purification plant, Egypt, and screened for their electrogenic potential. The most promising isolates were identified according to 16S rRNA sequencing as *Escherichia coli* and *Enterobacter cloacae*, promising results were achieved on using them in consortium at optimized values of pH (7.5), temperature (30℃) and substrate (glucose/pyruvate 1%). Low level red laser ($\lambda = 632.8$nm, 8mW) was utilized to promote the electrogenic efficiency of the bacterial consortium, maximum growth was attained at 210 sec exposure interval. In an application of adding standard inoculum ($10^7$ cfu/mL) of the photo-stimulated bacterial consortium to sludge based MFC a significant increase in the output potential difference values were recorded, the electricity generation was maintained by regular supply of external substrate. These results demonstrate the future development of the dual role of MFCs in renewable energy production and sludge recycling.

**Keywords**  Electrogenic Bacteria, Microbial Fuel Cell (MFC), Green Electricity, Sludge, pH, Temperature, photo-stimulation

## Introduction

Energy requirement is ever increasing throughout the world, electricity is considered the basic energy in our daily life. Seeking alternative cheap and ecofriendly sources of energy generation have become a must [1]. Microbial fuel cell (MFC) is considered a bio-electrical energy system that converts chemical energy contained in organic substrates into electrical energy by the microbial activities, so it acts as a promising sustainable inexpensive alternative for renewable energy generation [2], the efficiency of MFC technology comes from the capability of electrogenic bacteria to oxidize organic matter under anaerobic conditions [3], the oxidation process produces electrons and protons, the electrons released at the anode travel through an external electrical circuit to the cathode, creating a flow of electric current, while the protons move directly to the cathode through the solution [4]. Studies reported that transferring electrons to the anode can take place either by mediator electron transfer via an exogenous redox mediator [5] or by direct electron transfer as the microbe physically contacted to the anode forming a biofilm [6]. Traditional wastewater treatment methods faced significant difficulties from

*Correspondence:
Nashwa A. Ahmed
nashwa.abbas.ams@o6u.edu.eg
[1]Faculty of Applied Health Sciences Technology, October 6 University, P.O. Box 12585, El- Giza, Egypt
[2]Microbial Biotechnology Department, Genetic Engineering and Biotechnology Institute, Sadat City University, Sadat city, Egypt

high operational costs, high energy usage, and environmental pollution [7]. In addition to green energy production electrogenic microbes have enzymatic potential to degrade different macromolecules with high conversion efficiency compared to other electrochemical cells [8] Biodegradable substrates are considered as electron sources in the MFC, these substrates range from simple compounds to complex organic compounds [9], previous reports documented that sludge is used as a fuel for several anaerobic and facultative anaerobic bacterial species because of its nutrient richness and year-round availability. It is made up of effluents obtained from urban, industrial, and other sources, which are regarded as the primary source of energy to produce electricity [10]. Pure or mixed cultures of microorganisms can be used in MFC, however mixed cultures are more efficient energy generators [11]. Electrogenic microorganisms are obtained from widely used sources such as soil or marine sediment, the natural microbial population, and brewery wastewater are added to mixed cultures to increase biological constancy [12]. Suitable environmental conditions including pH, temperature and nutrients are required for the growth and multiplication of electrogenic bacteria [13]. Temperature and pH affect the activity of enzymes, which will have a great impact on the growth and reproduction of microorganisms. When the temperature is greater or lower than the optimum value, the intracellular enzymatic activity will decrease, thus affecting the power generation potentiality of the MFC [14], also environmental pH is closely related to the life activities and metabolic performance of electrogenic bacteria, so the power generation capacity of MFCs varies greatly under different pH conditions [15]. In addition, studies have reported that the lack or excess of nutrients in the growth environment of microorganisms will directly affect their metabolic activities consequently their electricity production [16], Kim et al [17] reported that the produced power was more efficient on using glucose compared to other substrates. Light biotechnology is a recent field that contributes to the studies related to the environment through the photo stimulation of the bioenergetic processes of microorganisms [18]. Photo-energizing systems include low level red laser that emits light with a relatively narrow spectral band at a certain wavelength [19], it is hypothesized that induction of the photo-modulatory microbial system by red laser promotes the increase in cellular biomass and protein synthesis [20], since this inducer tool is cost -effective so it is introduced in many environmental and medical applications.

The aim of the present work is to isolate, screen and identify the most potent electrogenic bacterial isolates from sludge samples, assess the effect of different environmental factors and different substrates on their growth, finally investigate the effect of photo-stimulation using low level red laser on the electrogenic potentiality of the bacterial consortium to introduce a new approach in boosting the efficiency of green electricity production.

## Material and methods
### Materials
All materials, chemicals and reagents are of analytical grade and were purchased from Sigma Aldrich.

### Sample collection
Sludge samples were collected throughout the year 2021 from El-Sheikh Zayed water purification plant, Giza, Egypt. Triplicate samples were collected in sterile one-liter bottles in January (S1), April (S2), July (S3) and October (S4), the samples were transported to the laboratory in a cooling box, where they immediately stored in a refrigerator at 4°C until processing within 24 h, different Physicochemical parameters had been carried out using inductively coupled plasma/Mass spectrometry (ICP/MS) (Thermo. Fisher, USA).

### Enumeration of total bacterial counts
For quantitative analysis, sludge samples were vortexed for 3 mins to be completely homogenous, one mL of each sample was suspended separately in 9 mL of sterile saline, a series of decimal dilutions were prepared, 0.1 mL of each sample was plated on nutrient agar medium and incubated for 24 h at 37°C, total viable count (TVC) was expressed as colony forming units (cfu/mL) of the sample, experiments were undertaken in triplicates [21].

No. of cfu/mL=No. of colonies counted × Dilution factor /Volume of sample taken

### Construction of the microbial fuel cell (MFC) system
Single chambered Microbial fuel cell (MFC) was implemented in a rectangular plastic chamber with an inner working volume of one liter to suit the amount of the sample and the size of the electrodes used. The MFC was equipped with two graphite rods to serve as the two electrodes (anode and cathode) with an active area 25cm$^2$. A positive heavy load clamp wire was used to be connected to the anode and another negative one is connected to the cathode (The cathode electrode was coated with Poly tetrafluoroethylene (PTFE) diffusion layers on the air-exposed side; 60% w/v, dispersion in water). These two electrodes were placed on opposing sides and were connected to a digital multi-meter (model number AVO DT-9205A) manufactured by SUNDER Electronics, India (Fig. 1) through an external circuit across a resistance of 500 Ω. The digital multi-meter is assigned to measure the Potential difference (mV) produced by the MFCs [22].

**Fig. 1** Schematic diagram of the microbial fuel cell

### Measurement of the electric potential in the collected sludge samples

The constructed MFCs were fed with 500 mL of each sludge sample diluted with phosphate buffer solution (PBS) at the ratio of 4:1, the experiments were conducted at constant temperature the digital multi-meter's two wires were secured to the cathode and anode poles, and the readings were recorded along ten days of operation at regular intervals [23].

### Isolation, purification, and primary identification of the isolated bacterial strains

Morphologically distinct colonies were picked, isolates were purified by streaking on nutrient agar, pure cultures were maintained at 4ºC, different bacterial isolates were identified using the VITEK® MS (bioMérieux, Marcy-l'Etoile, France), an automated microbial identification system using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and the biochemical-based VITEK®2 system (bioMérieux, Marcy-l'Etoile, France) [24].

### Screening of the most potent electrogenic bacterial isolates

For the selection of the most potent electrogenic bacterial isolates, the constructed MFCs were sterilized via rinsing with 70% Ethanol and UV irradiated for 30 min, they were seeded with the prepared basal medium (BM) using the following constituents (g/L deionized water): glucose, 3 ; $NaHCO_3$, 2.5; $NH_4Cl$, 0.2 ; $KH_2PO_4$, 0.42; KCl, 0.33; NaCl, 0.3; $K_2HPO_4$, 1.26; $CaCl_2.2H_2O$, 0.15; $MgCl_2$, 3.15; yeast extract, 1. Ten mL of mineral media were added to the previous constituents. The mineral media consists of (g/L deionized water): EDTA, 0.5; $CoCl_2.6H_2O$, 0.082; $CaCl_2.2H_2O$, 0.114; $H_3BO_3$, 0.01; $Na_2MoO_4.2H_2O$ 0.02; $Na_2SeO_3$, 0.001; $Na_2WO_4.2H_2O$,

0.01; $NiCl_2.6H_2O$, 0.02; $MgCl_2$, 1.16; $MnCl_2.4H_2O$, 0.59; $ZnCl_2$, 0.05; $CuSO_4.5H_2O$, 0.01; $AlK(SO_4)_2$, 0.01. The pH value was adjusted at 7 using NaOH solution. The pure six bacterial isolates were reinoculated in sterile nutrient broth separately and incubated for 24 h at 37°C [20], a standard fresh inoculum ($10^7$ CFU/mL) of each bacterial isolate was prepared, cultures' inocula were distributed in the separate chambers of MFCs, and electricity generation was measured every 24 h during a period of five days.

### Molecular identification of the most potent bacterial isolates by 16S RNA

The bacterial isolates were cultured on 5 mL brain heart infusion broth at 37°C for 24 h, the grown cells were collected by centrifugation at 12.000 rpm for 2 mins for DNA extraction. The extracted DNA was then used as a template for 16S rRNA gene amplification. through polymerase chain reaction (PCR) using Maxima Hot Start PCR Master Mix (Fermentas). The universal primers used for the amplification were F: 5'AGAGTTTGATCCTG-GCTCAG'3 & R: 5'GGCTACCTTGTTACGACTT'3. The PCR reaction was performed according to the recommended thermal cycling conditions as follows: denaturation at 95°C for 5 mins (1 cycle), denaturation at 95°C for 30 sec, annealing for at 56°C 30 sec, extension at 72°C for 30 sec, and (40 cycles), and final extension at 72°C for 10 min (1 cycle). Following that, the PCR products were loaded onto a 1.5 percent agarose gel (Biometra, Germany) and electrophoresed in 1 X TBE solution at 100V for approximately 30 mins, it was then dyed with 2µl of 10mg/ml ethidium bromide (Sigma, USA). UV transilluminator (UVP-dual-Intensity Transilluminator,

MultiDoc-It™ system) with wavelength 312nm was used to visualize DNA samples, and a UVP-gel documentation system (DigiCam 130) was used to picture them.

Sequencing was performed using the ABI 3730xl DNA sequencer following the manufacturer's instructions. The sequence was submitted to the database of the National Center for Biotechnology Information (NCBI) GenBank (http://www.ncbi.nlm.nih.gov/BLAST) and compared to other available sequences, and a phylogenetic tree was constructed by the MEGA software version 6 [25].

### Optimization of bacterial growth conditions

Triplicate screw capped 250 mL bottles containing 100 mL of the prepared BM were adjusted at different pH values (6, 6.5, 7, 7.5, 8,) using 1 mol/L HCl and NaOH solutions. The media were inoculated with the selected electrogenic bacterial isolates individually ($10^7$ cfu/mL), after 48 h of incubation at 37°C, 100 µL from each bottle was spread on nutrient agar plates, the TVC was recorded (cfu/mL) after 24h of incubation at 37°C and the optimum pH was recorded. For temperature adjustment, triplicate screw capped 250mL bottles containing 100 ml of the prepared BM adjusted at the optimum pH were inoculated ($10^7$ cfu/mL), after 48h of incubation at different temperatures (20°C, 25°C, 30°C, 35°C, 40°C) the TVC results were recorded as reported before and the optimum temperature was detected. The impact of various substrates on the growth of each electrogenic bacterial isolate was evaluated as the previous experiment by replacing the electron donner (Glucose) with the same concentration of different substrates; Lactose, Fructose and Pyruvate individually and in mixtures, growth results were recorded as cfu/mL, and the optimum substrate was determined [26].

### Assessment of electrogenic potentiality of the selected bacterial isolates individually and in consortium at optimum conditions

MFCs were constructed, stuffed with 500 mL BM medium supplemented with 1% Glucose/Pyruvate as substrate, the optimum growth conditions were maintained before seeding with a standard bacterial inoculum of the two most potent electrogenic bacterial isolates individually and in consortium ($10^7$cfu/mL). Electricity generation was measured in terms of output potential difference (mV) with a multi-meter along twelve days with regular intervals. Every 3 days interval 100 mL of 1% substrate was added in the cell after discarding similar volume, to ensure continuous cell operation [27].

### Photo stimulation of electrogenic bacterial consortium

Photo stimulation of the selected electrogenic bacterial consortium was carried out in the National Research Centre (NRC), Egypt using low level red laser (LLRL) tube (ThORLABS, USA) at wave length of 632.8 nm and output power 8 mW, an adjustable stand was used to stabilize the laser tube at 20mm height, irradiation takes place in dark room at 30±2°C, the bacterial suspension of the bacterial consortium at optimum conditions was subjected to different radiation doses via different exposure intervals (0, 30, 60, 90, 120, 150, 180, 210, 240 sec), the standard bacterial suspensions were inoculated in triplicate screw capped 250mL bottles containing 100mL BM medium, after 24h of incubation at 30°C the TVC was recorded as cfu/mL as in the optimization step [28].

### Operation of natural sludge based MFC using photo-stimulated bacterial consortium

MFCs were constructed, stuffed with 500 mL sludge diluted with PBS at the ratio of 4:1, the optimum growth conditions were maintained before seeding with a standard inoculum ($10^7$cfu/mL) of the irradiated and non-irradiated bacterial consortium individually, Electricity generation was measured in terms of potential difference (mV) with a multi-meter along twelve days with regular intervals. Every 3 days interval 100 mL of 1% substrate was added in the cell after discarding similar volume, to ensure continuous cell operation [27].

### Statistical analysis

Recorded data were analyzed using the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). The quantitative data were presented as mean±standard deviation and ranges, also qualitative variables were presented as numbers and percentages. A one-way analysis of variance (ANOVA) when comparing between more than two means & Post Hoc test: Tukey's test was used for multiple comparisons between different variables. Repeated measures analysis of variance (ANOVA) was used to compare multiple within-group measures. The Bonferroni correction was used to adjust the p-value for multiple within-group comparisons.

## Results

### Physicochemical parameters of sludge samples

Four sludge samples were obtained along the period from January 2021 till October 2021 (S1-S4) from El-Shiekh Zayed water purification plant, Giza, Egypt. Physicochemical parameters of the collected sludge samples were recorded (Table 1), results revealed the presence of different metals and ions in the four samples with slight fluctuation in the mean concentration values, also the results of measurement of turbidity, total dissolved solids and electric conductivity were recorded, higher results were detected in S3 sample. The two analytical parameters biological oxygen demand (BOD), and chemical oxygen demand (COD) were estimated, our results showed that compared to other samples S3 had higher COD and BOD values and lower COD/BOD ratio (<2).

**Table 1** Physicochemical analysis of the collected sludge samples

| Analysis type | Unit | Samples | | | |
|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S4 |
| Turbidity | NTU | 4000 | 5800 | 18462 | 4500 |
| Temperature | °C | 22.4 | 15.5 | 27.6 | 25.2 |
| pH | - | 6.9 | 7.4 | 7.3 | 7.0 |
| TDS | mg/l | 310 | 320 | 340 | 308 |
| Electric conductivity | Us/cm | 470 | 512 | 530 | 465 |
| Total alkalinity | mg/l | 612 | 480 | 130 | 530 |
| Total hardness | mg/l | 520 | 540 | 150 | 350 |
| Permanent hardness | mg/l | 92 | 60 | 20 | 50 |
| Temporary hardness | mg/l | 520 | 480 | 130 | 358 |
| Calcium hardness | mg/l | 416 | 300 | 102 | 250 |
| Magnesium hardness | mg/l | 104 | 240 | 48 | 180 |
| F | mg/l | 2.0 | 0.3 | 2.5 | 0.2 |
| $SO_4^2$ | mg/l | 40.0 | 39.0 | 50.0 | 35.0 |
| $PO_4$ | mg/l | 0.025 | 19 | 5.98 | 18.0 |
| $NO_2$ | mg/l | 0.002 | 0.01 | 0.05 | 0.01 |
| $NO_3$ | mg/l | UDL | UDL | UDL | UDL |
| Ca | PPM | 166.0 | 120.0 | 40.8 | 118.0 |
| Mg | PPM | 25 | 57.6 | 11.52 | 55.0 |
| Al | PPM | 0.31 | 3 | 0.62 | 0.55 |
| Fe | PPM | 0.18 | 8.03 | 2.8 | 0.36 |
| Li | PPM | 0.004 | 0.20 | 0.426 | 0.005 |
| B | PPM | 2.899 | 1.8 | 4.295 | 2.0 |
| Na | PPM | 143.042 | 122.0 | 106.124 | 111.0 |
| K | PPM | 1.015 | 5.0 | 6.914 | 4.0 |
| Ti | PPM | 2.474 | 8.0 | 19.640 | 2.1 |
| Cr | PPM | 0.099 | 1.2 | 0.472 | 0.9 |
| Mn | PPM | 0.306 | 1.3 | 18.163 | 0.5 |
| Co | PPM | 0.003 | 1.5 | 6.562 | 1.8 |
| Ni | PPM | 0.023 | 1.3 | 0.480 | 1.5 |
| Cu | PPM | 0.013 | 5.2 | 11.086 | 2.2 |
| Zn | PPM | 0.336 | 4.4 | 7.839 | 4.2 |
| Ga | PPM | 0.006 | 0.01 | 0.633 | 0.5 |
| Sr | PPM | 0.079 | 0.6 | 6.914 | 0.2 |
| Ag | PPM | 0.004 | 1.2 | 6.968 | 0.8 |
| Cd | PPM | 0.000 | 1.3 | 9.640 | 0.9 |
| In | PPM | 0.000 | 0.1 | 0.472 | 0.1 |
| I | PPM | 1.263 | 2.0 | 8.163 | 1.9 |
| Ba | PPM | 0.186 | 1.0 | 2.649 | 0.9 |
| Hg | PPM | 0.017 | 0.11 | 0.562 | 0.2 |
| Pb | PPM | 0.009 | 0.02 | 0.480 | 0.1 |
| Bi | PPM | 0.008 | 0.3 | 1.086 | 0.01 |
| BOD | PPM | 56.0 | 55.0 | 110 | 35.0 |
| COD | PPM | 120 | 112.0 | 198 | 100.0 |
| COD/BOD | - | 2.1 | 2.03 | 1.8 | 2.8 |

### Enumeration of total bacterial counts

The mean numerical values of total viable bacterial counts were detected for the four collected sludge samples, the results represented in Fig. 2 showed highly statistically significant value of total viable count ($1.8 \times 10^7$ cfu/mL) in S3 sample (p-value < 0.001), while the lowest value was detected in S1 sample ($0.1 \times 10^7$ cfu/mL).

### Detection of electrogenic bacteria from the collected sludge samples

To detect the presence of electrogenic bacteria in the collected sludge samples, single chambered sludge based MFCs were fabricated, the potential differences were measured along 10 days of operation (Table 2), the results indicated that the maximum measured output voltage was ranged from 281.93 to 430.80 mV. For the four samples the electricity generation passed through four stages, stage one which represents the rapid fall in the output voltage, stage two the output voltage remains nearly stable, stage three at which there was a rapid fall in the output voltage, finally stage four which represented a nearly steady state of low voltage.

### Isolation and primary identification of bacterial strains

Six distinct bacterial isolates were isolated from S3 sludge sample, the bacterial isolates were purified and primary identified using VITEK®2 system which permits rapid identification and antimicrobial susceptibility testing of the bacteria, the results illustrated in Table 3 showed that the six isolates were identified as *Aeromonas salmonicida* (*A. salmonicida*), *Pantoea sp, Enterobacter cloacae* (*E. cloacae*), *Staphylococcus lentus* (*S. lentus*), *Comamonas testosterone* (*C. testosterone*) and *Escherichia coli* (*E. coli*).

### Screening and molecular identification of the most potent electrogenic bacterial isolates

Synthetic growth medium was prepared to be used as a nutrient supplement to enhance the growth and oxidation power for electrogenic bacteria, results represented in Table 4 revealed that among the six bacterial isolates, five were found to have electrogenic behavior, as no readings were recorded for *Pantoea* sp. *E. cloacae* and *E. coli* were selected to be significantly the most potential based on the measurements of the output potential difference along a period of five days.

### Molecular identification of the most potent bacterial isolates by 16S RNA

The most potent electrogenic bacteria (*E. cloacae* and *E. coli*) were subjected to confirmatory molecular characterization by using the 16S ribosomal RNA (16S rRNA) marker gene. The Basic Local Alignment Search Tool (BLAST) homology search and motif analysis revealed that the strains were located in the positions occupied by the genera *Enterobacter* and *Escherichia* Fig. 3 declared the results of the phylogenetic characterization and showed that strains NR_028912.1 and NR_112558.1 were most likely related to the strains *E. cloacae* and *E. coli* respectively.
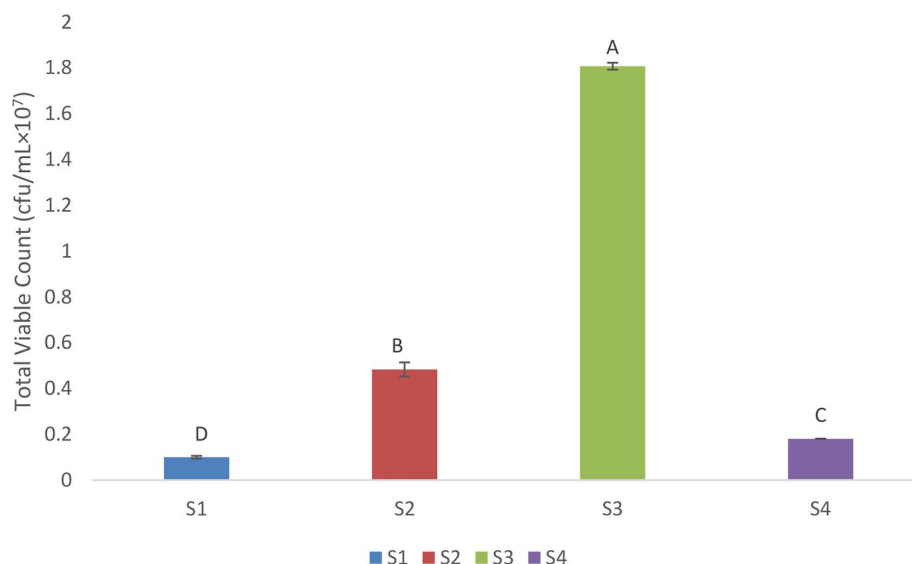
**Fig. 2** Total viable bacterial count (cfu/mL) in the collected sludge samples. One way Analysis of Variance test was performed for Mean±SD & Multiple comparison between groups through Post Hoc test: Tukey's test Letters A-D comparatively represent the descending order of results reflecting importance among means. p-value > 0.05 is insignificant; *p-value < 0.05 is significant; **p-value < 0.001 is highly significant

**Table 2** Output potential difference values in the collected sludge samples

| Time (Days) | | Output Potential difference values (mV) | | | | F-test | p-value |
|---|---|---|---|---|---|---|---|
| | | **S1** | **S2** | **S3** | **S4** | | |
| Day 0 | Stage One | 281.93±0.83D | 365.23±0.25B | 430.80±0.98A | 297.00±0.50C | 49.539 | <0.001** |
| Day 1 | | 177.87±0.78D | 262.50±0.50B | 310.90±0.66A | 189.50±0.50C | 53.756 | <0.001** |
| Day 2 | Stage Two | 150.80±0.62C | 200.60±0.66B | 270.53±0.45A | 156.70±0.44C | 68.468 | <0.001** |
| Day 3 | | 146.83±0.47C | 198.50±0.50B | 250.67±0.40A | 151.90±0.26A | 23.658 | <0.001** |
| Day 4 | | 133.80±0.62D | 182.00±0.90B | 211.00±0.20A | 147.87±0.51C | 33.974 | <0.001** |
| Day 5 | Stage Three | 84.43±0.31D | 105.93±0.60B | 136.23±0.25A | 92.63±0.45C | 20.381 | <0.001** |
| Day 6 | | 63.30±0.26D | 75.37±0.47B | 98.37±0.25A | 71.53±0.40C | 49.050 | <0.001** |
| Day 7 | | 31.33±0.15D | 43.70±0.44B | 50.70±0.46A | 38.67±0.35C | 79.031 | <0.001** |
| Day 8 | Stage Four | 28.17±0.15D | 41.83±0.76B | 48.67±0.50A | 34.77±0.32C | 20.399 | <0.001** |
| Day 9 | | 26.23±0.25D | 37.47±0.45B | 46.40±0.26A | 31.63±0.35C | 35.520 | <0.001** |
| Day 10 | | 24.30±0.20D | 35.53±0.55A | 30.23±26.19A | 29.27±0.25C | 33.627 | <0.001** |

Data are expressed by Mean±SD & Multiple comparison between groups through Post Hoc test: Tukey's test.

Letters A-D comparatively represent the descending order of results reflecting importance among means in the same row p-value>0.05 is insignificant; *p-value<0.05 is significant; **p-value<0.001 is highly significant

DNA fragments of genes of the Egyptian strains *E. coli* and *E. cloacae* were subjected to DNA sequencer to reveal their internal structure. Sequence alignment of 16S rRNA genes were done on the GenBank Database on the World Wide Web (see supplementary Fig S1 online), for *E. coli* gene the results illustrated sixty-nine types of mutations including substitution (inversion and transversion mutation), deletion and insertion mutations. Inversion mutations were detected at positions Q20 Q268 Q321 (G>A), Q21 Q47 Q64 Q73 Q82 Q95 Q121 Q131 Q133 Q153 Q252 Q304 Q352 (A>G), Q128 (C>T) and Q237 (T>C). At the same time transversion mutations were recorded at positions Q3 Q69 Q111 Q115 Q266 Q276 Q 290 Q357 (G>T), Q9 Q168 Q205 Q260 Q341 (T>G), Q17 Q52 Q58 Q90 Q107 Q158 Q273 (C>G), Q67 (A>C), Q78 Q137 Q140 Q248 Q340 Q360 (T>A), Q88 Q173 Q185 Q315 (C>A) and Q105 Q174 Q201 (A>T). Deletion mutations were detected at between positions Q34-35 (-T), Q114-115, Q315-316 (-A), Q135-136, Q281-282, Q315-316 (-G) and Q266-267 (-C). At the same time insertion mutations were recorded at positions Q38 Q295 (+T), Q144 (+G), Q279 Q301 Q328 (+A) and Q323 Q324 Q325 Q353 (+C). For *E. cloacae* there was one type of mutation including substitution mutation (inversion mutation) which was detected at position Q802 (C>T) (see supplementary Table S2 online).

**Table 3** Identification of the isolated bacterial strains by VITEK® 2 System

| Test | Mnemonic | A. salmonicida | Pantoea sp. | E. cloacae | S. lentus | C. testosterone | E. coli |
|---|---|---|---|---|---|---|---|
| Ala-Phe-Pro Arylamidase | APPA | - | - | - | - | - | - |
| L-Pyrrolidonyl Arylamidase | PyrA | - | - | - | - | + | - |
| Beta-Galactosidase | BGAL | - | - | + | - | - | + |
| D-Glucose | dGLU | - | + | + | - | - | + |
| ᵞ Glutamyl Transferase | GGT | - | - | + | - | - | + |
| Fermentation/Glucose | OFF | - | - | + | - | - | + |
| Beta-Glucosidase | BGLU | - | + | - | - | - | + |
| D-Maltose | dMAL | - | + | + | - | - | + |
| D-Mannitol | dMAN | - | + | + | + | - | + |
| D-Mannose | dMNE | - | + | + | + | - | + |
| Beta-Xylosidase | BXYL | - | - | + | - | - | + |
| Beta-Alanine arylamidase pDNA | BALap | - | - | - | - | - | + |
| L-Proline Arylamidase | ProA | + | - | - | - | - | - |
| Lipase | LIP | + | - | - | - | - | - |
| Palatinose | PLE | - | - | + | - | - | - |
| Tyrosine Arylamidase | TyrA | - | - | + | - | + | - |
| Urease | URE | - | - | - | - | - | - |
| D-Sorbitol | dSOR | - | - | + | - | - | - |
| Sucrose | SAC | - | - | + | - | - | + |
| D-Tagatose | dTAG | - | - | - | - | - | + |
| D-Trehalose | dTRE | - | + | + | - | - | + |
| Citrate | CIT | - | - | + | - | - | - |
| Malonate | MNT | - | + | + | - | - | - |
| 5-Keto-D-Gluconate | 5KG | - | - | - | - | - | + |
| Alpha-Glucosidase | AGLU | - | - | - | - | - | - |
| Succinate Alkalinization | SUCT | - | - | + | - | + | - |
| Beta-N-Acetyl-Galactosaminidase | NAGA | - | - | + | + | - | - |
| Alpha-Galactosidase | AGAL | - | - | + | - | - | - |
| Phosphatase | PHOS | - | - | - | + | - | + |
| Glycine Arylamidase | GlyA | - | - | + | - | - | - |
| Ornithine Decarboxylase | ODC | - | - | + | - | - | + |
| Lysine Decarboxylase | LDC | - | - | - | - | - | + |
| Courmarate | CMT | - | - | + | - | - | - |
| Beta-Glucuronidase | BGUR | - | - | - | - | - | + |
| Glu-Gly-Arg- Arylamidase | GGAA | - | - | - | - | + | - |
| Ellman | ELLM | - | - | - | - | + | - |

**Table 4** Electrogenic potentials of the isolated bacterial strains

| Time (Days) | Potential difference (mV) | | | | |
|---|---|---|---|---|---|
| | A. salmonicida | E. cloacae | S. lentus | C. testosterone | E. coli |
| One | 28.47 ± 0.81C | 112.77 ± 0.31A | 20.60 ± 0.78E | 22.83 ± 0.25D | 98.73 ± 0.38B |
| Two | 30.13 ± 1.06C | 115.93 ± 0.67A | 21.87 ± 0.67D | 24.17 ± 0.31D | 100.47 ± 0.25B |
| Three | 32.23 ± 1.16C | 118.73 ± 0.68A | 26.33 ± 0.31D | 26.80 ± 0.30D | 104.07 ± 0.45B |
| Four | 20.47 ± 0.60C | 90.50 ± 0.53A | 15.53 ± 0.35E | 16.73 ± 0.40D | 65.90 ± 0.44B |
| Five | 16.47 ± 0.65C | 74.33 ± 0.59A | 11.33 ± 0.47E | 14.40 ± 0.26D | 45.77 ± 0.42B |
| **RMANOVA** | 174.687 | 3434.438 | 320.862 | 938.691 | 1297.205 |
| **p-value** | < 0.001** | < 0.001** | < 0.001** | < 0.001** | < 0.001** |

Repeated-measures analysis of variance (ANOVA) & Multiple comparison between groups through Post Hoc test: Bonferroni-corrected. Letters A-E comparatively represent the descending order of results reflecting importance among means at the same time. $p$-value $> 0.05$ is insignificant; *$p$-value $< 0.05$ is significant; **$p$-value $< 0.001$ is highly significant
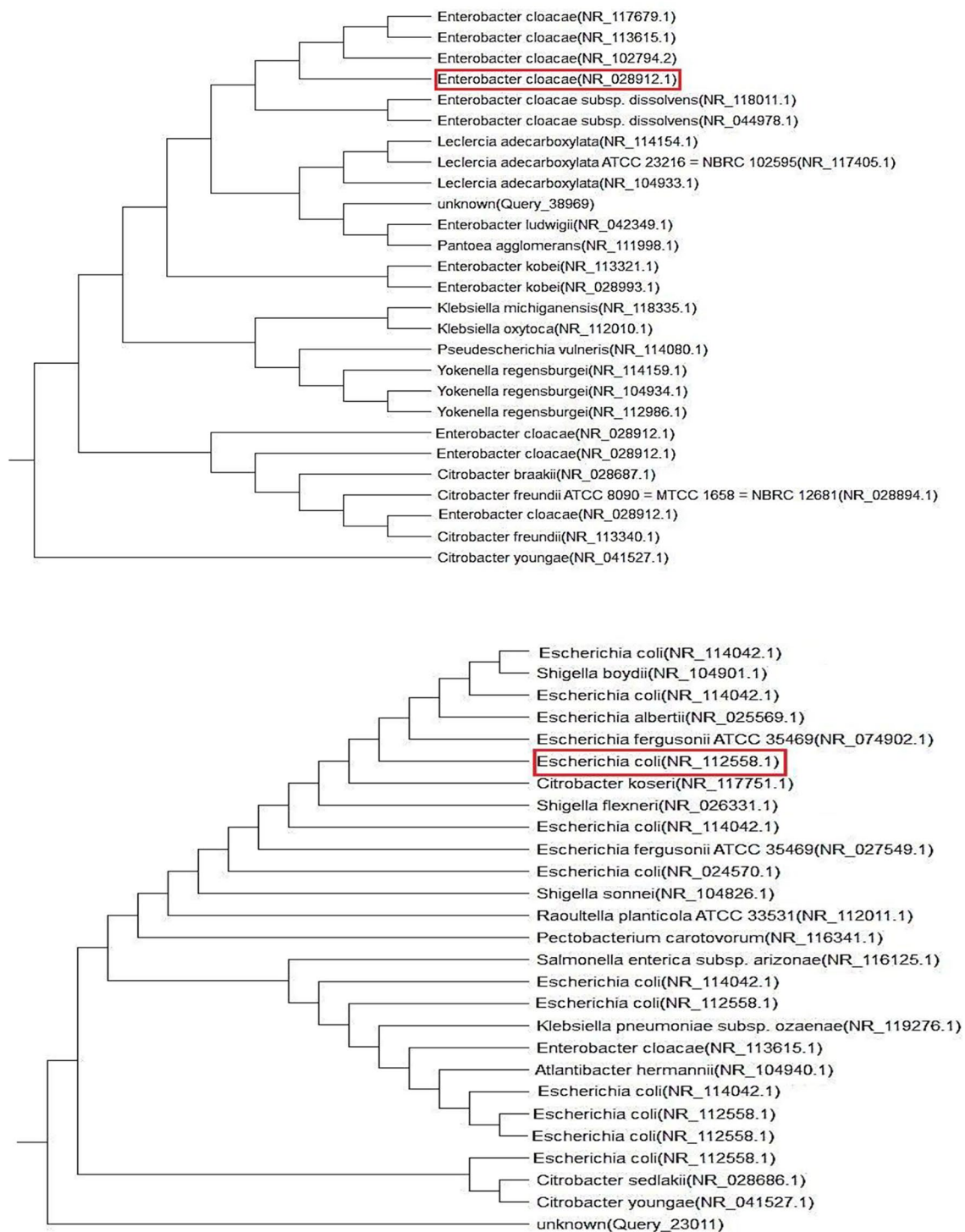
**Fig. 3** Phylogenetic tree of *E. coli and E. cloacae* based on 16S rRNA constructed using the neighbor joining method.

## Optimization of the growth conditions of the most potent electrogenic bacteria

As shown in Fig. 4 the statistically significant higher mean values of total viable counts (cfu/mL) were recorded at pH 7.5 and temperature 30°C for both *E.*

*cloacae* and *E. coli* with p-value (p<0.001) so they are considered the most optimum and their values are taken for further studies.

The results represented in Fig. 5 revealed statistically significant higher mean value of total viable counts (cfu/
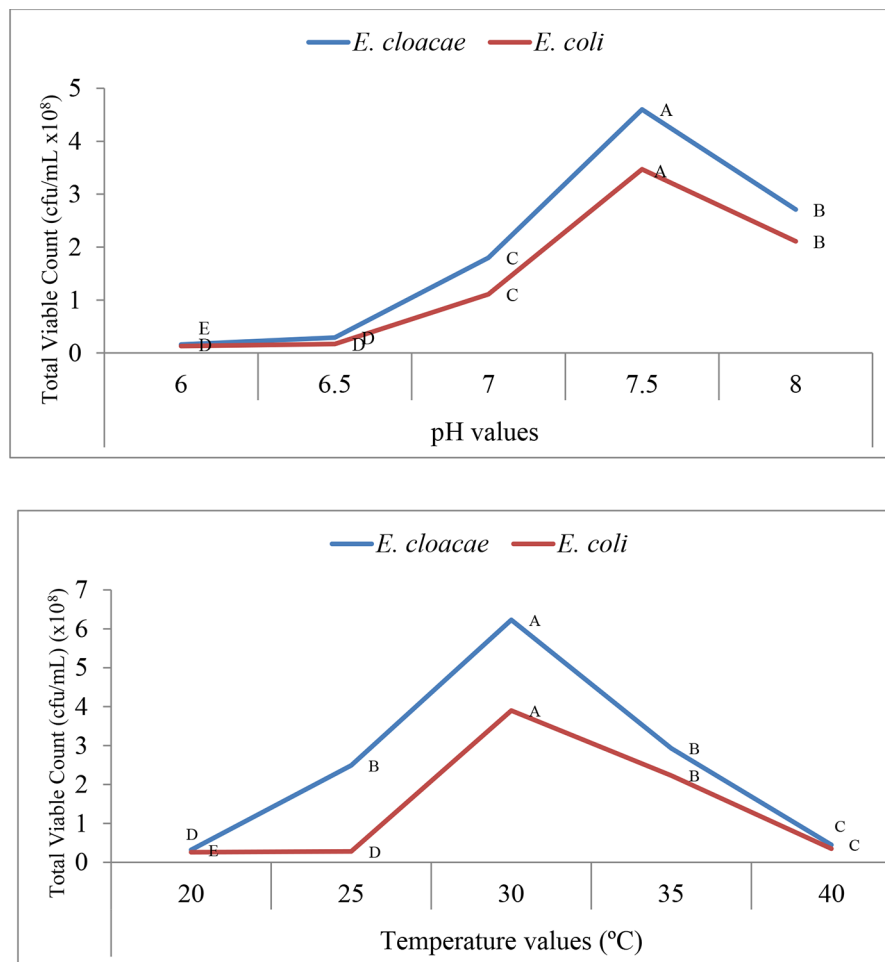
**Fig. 4** Total viable count (cfu/mL) at different ph and temperature values. Repeated-measures analysis of variance (ANOVA) & Multiple comparison between groups through Post Hoc test: Bonferroni-corrected. Letters A- E comparatively represent the descending order of results reflecting importance among means at the same value, p-value > 0.05 is insignificant; *p-value < 0.05 is significant; **p-value < 0.001 is highly significant

mL) for *E. cloacae* and *E. coli* were recorded on using glucose/ pyruvate with p-value (p<0.001).

**Electrogenic potentiality of the selected bacterial isolates individually and in consortium at optimum conditions**
MFC loaded with synthetic media at optimum conditions was used to find out the electrogenic potentiality of *E. cloacae* and *E. coli* individually and in consortium, electricity generation in terms of potential difference (mV) was recorded using multimeter from day one and extends throughout twelve days, the results represented in Fig. 6 declared that for both isolates and their consortium the electricity generation was in direct proportion with time till day three at which the recorded values started to decrease due to depletion of the substrate, on supplying the MFC with 1% Glucose/Pyruvate at regular intervals, the mean values of potential difference started to rise, the results showed statistically significant higher mean value of Potential difference (mV) in bacterial consortium

compared to both isolates individually at each time (p<0.001).

**Photo stimulation of electrogenic bacterial consortium**
Our results clarified a dose dependent stimulatory effect of LLRL on the growth rate of the bacterial consortium (Fig. 7), significant maximum population was recorded at 210 sec of irradiation ($13.5 \times 10^8$ cfu/mL value (p<0.001), on the other hand increasing the irradiation interval resulted in photoinhibition leading to significant decrease in the TVC.

**Operation of natural sludge based MFC using photo-stimulated bacterial consortium**
Sludge based MFC was constructed as a kind of application to use sludge obtained from chemical precipitation, sedimentation or any other process in the generation of green electricity, our results (Fig. 8) declared that addition of exogenous bacterial inoculum of wild and photo-stimulated bacterial consortium showed high
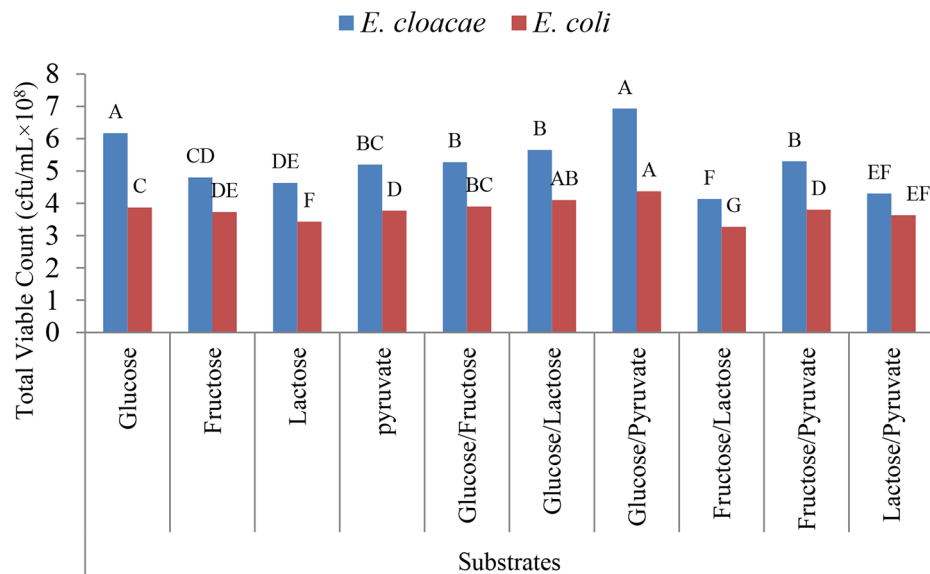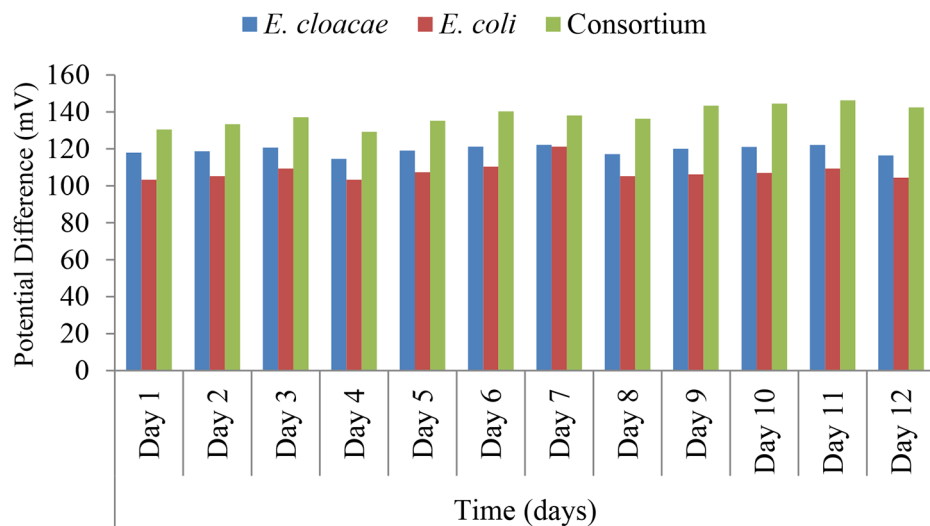
■ *E. cloacae*   ■ *E. coli*



**Fig. 5** Total viable count (cfu/mL) using different types of substrates. Repeated-measures analysis of variance (ANOVA) & Multiple comparison between groups through Post Hoc test: Bonferroni-corrected. Letters A-F comparatively represent the descending order of results reflecting importance among means at the same value, p-value > 0.05 is insignificant; *p-value < 0.05 is significant; **p-value < 0.001 is highly significant

■ *E. cloacae*   ■ *E. coli*   ■ Consortium



**Fig. 6** Pattern of potential difference output for *E. cloacae, E. coli* and their consortium. Paired Sample t-test. p-value > 0.05 is insignificant; *p-value < 0.05 is significant; **p-value < 0.001 is highly significant

synergistic action, leading to increasing the performance of the MFC, there was a statistically significant higher mean value of potential difference (mV) in photo-stimulated bacterial consortium compared to wild bacterial consortium in each time (p<0.001), the addition of fresh medium containing glucose/pyruvate (1%) at regular intervals over comes the depletion of nutrients and led to the revival of the endogenous and exogenous microbial flora, thus the mean value of the potential difference was maintained during the time of operation.

## Discussion

Sludge production volumes have been increased steadily every year [29]. Major issues arise when large amounts of sludge are dumped into the environment, including worsened odors, an increased risk of pathogenic microorganisms, and heavy metal accumulation [30]. In the present study four sludge samples acting as a microbial source were obtained from El-Shiekh Zayed water purification plant, the higher recorded results of the concentrations of metals and ions detected in S3 sample might be due to the rain effect which causes soil erosion, so
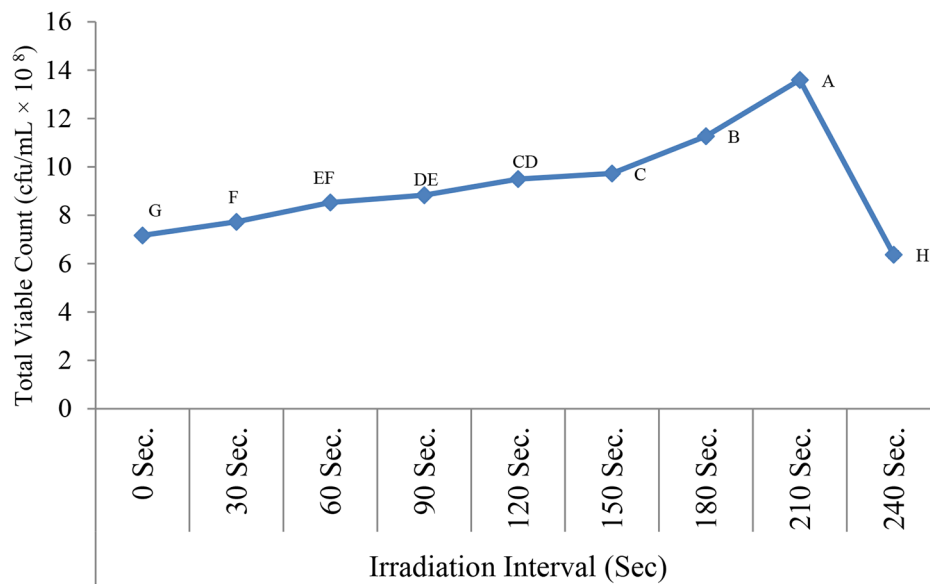
**Fig. 7** Effect of LLRL on the bacterial growth of the electrogenic bacterial consortium. Repeated-measures analysis of variance (ANOVA) & Multiple comparison between groups through Post Hoc test: Bonferroni-corrected, letters A-H comparatively represent the descending order of results reflecting importance among means at the same value. p-value > 0.05 is insignificant; *p-value < 0.05 is significant; **p-value < 0.001 is highly significant
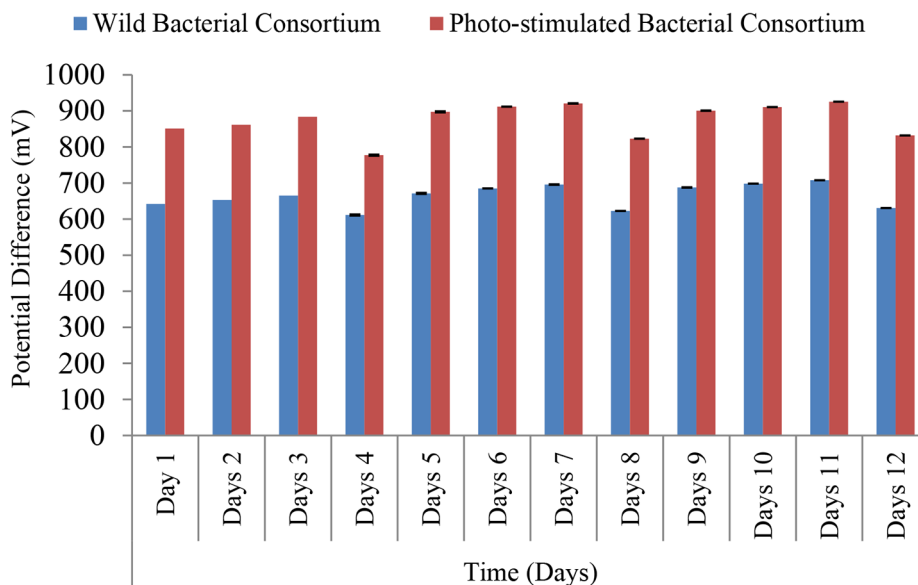


**Fig. 8** Pattern of potential difference using photo-stimulated bacterial consortium. Paired Sample t-test. p-value > 0.05 is insignificant; *p-value < 0.05 is significant; **p-value < 0.001 is highly significant

different elements were then transported to the combined sanitation network and dumped into the water treatment plant as described by Brahimi et al. [31], also the higher values of turbidity and electric conductivity reflects higher microbial load as reported by Mucha and Kułakows [32]. The two analytical parameters biological oxygen demand (BOD), and chemical oxygen demand (COD) reflected the oxygen content possibly present due to pollutants in the investigated sludge samples, BOD determines the amount of dissolved oxygen consumed by bacteria to oxidize organic compounds, while COD is an indicative measure of the amount of oxygen needed for complete oxidation of organic compounds [33], higher BOD and COD values reveled higher concentration of organic substrates needed for the persistent microflora, additionally low COD/BOD ratio (<2) indicated high performance of oxidation-reduction reactions by the naturally occurring microflora [34]. Temperature affected

the physicochemical parameters of sludge samples, so it is considered as a key factor in shaping the structures of microbial populations. High records of microbial loads of S3 sample are harmonious with the results of Madni et al [35] who reported that high temperature favors the survival of microorganisms. The global energy crisis and the limited resources necessitates the search for alternative sustainable energy sources. in MFCs the organic matter present in the sludge samples was subjected to biochemical conversion system to produce electric current [36], the electrogenic microflora acts as a catalyst that oxidizes the organic matter resulting in the release of electrons and protons, electrons were migrated to the anode and consequently transferred to the cathode through an external wire, while the protons transferred directly to the cathode through the solution, the created potential difference can be recorded [37]. The measured values of potential difference (mV) indicated that the microflora in all samples have electrogenic potential and they can manifest this potential if they are properly re-inoculated into their habitat, the electricity generation passes through four stages initiated by a rapid fall in the output voltage, then a nearly stable interval of electricity generation, followed by another rapid fall in the output voltage, finally a stage of nearly steady state of low voltage. Similar electricity generation pattern was recorded by Cau et al [2], the highly statistically significant results recorded for S3 samples are in correspondence to that of the TVC results. The isolation of six bacterial strains from the selected sludge sample declared the fact that electrogenic bacteria are present in all ecosystems [38], with different electrogenic potentialities due to their multiple reducing mechanisms [39], primary and rapid identification using VITEK®2 system showed that our isolated bacterial collection included *A. salmonicida* which is associated with fish [40], *S. lentus, E. cloacae*, and *E. coli* which are associated with animals [41], *C. testosterone* and *Pantoea sp* which are mainly found in soil, waste water and sludge [42], in agreement with the results recorded by Nguyena et al [43] the most potent electrogenic isolates were *E. cloacae*, and *E. coli*, consequently they were subjected to confirmatory molecular characterization by using the 16S rRNA marker gene which has served as an important tool for determining phylogenetic relationships between bacteria. [44]. BLAST was applied to find regions of similarity between biological sequences and the results of phylogenic characterization showed that the strains NR_028912.1 and NR_112558.1 were most likely related to the strains *E. cloacae* and *E. coli* respectively,  the sequence alignment of 16S rRNA gene illustrated the presence of some mutations including substitution (inversion and transversion mutation), deletion and insertion in both isolates. Generation of green electricity depends on the massive growth of electrogenic bacteria

which carry out oxidation reduction reactions to generate electric current, growth conditions play a significant role in the biomass production of the microorganisms that are capable of converting pollutants to electric current via electrochemical reactions [45]. There are many physical, chemical and biological parameters that affect the operation of MFCs [46]. In our study the optimum conditions studied for isolated electrogenic bacterial isolates (*E. cloacae* and *E. coli*) were evaluated by determination of the total viable bacterial count in terms of cfu/mL as their increase reflects the ability of substrate utilization and consequently increasing electrons flow rate [26]. pH value is one of the crucial factors that significantly impact the bacterial growth rate, it affects the catalytic ability of enzymes, and it also alters the ionic charge of many biomolecules affecting the metabolic reactions [47]. for both *E. cloacae* and *E. coli* was pH 7.5 was reported as the optimum pH value at which maximum total viable count was achieved. Near results were obtained by Kim et al. [17] who reported that the optimum pH range for the growth of Enterobacteriaceae was between 6 and 8. Temperature affects the spatial arrangement of the atoms in the biomolecules and hence at the optimum temperature the biomolecules retain a stable and active structure which enables it to carry out its function. At low temperatures, enzymes stop functioning as low temperatures increase the viscosity of fluids and hardening of lipids, while high temperatures cause rupture of hydrogen bonds in proteins and in DNA resulting in protein denaturation [48]. It was evident from our results that the optimum incubation temperature at which the maximum population was 30°C. These results are consistent with those reported by Sihag et al [49] who indicated that most bacteria are active in the mesophilic range of 25°C to 40°C. Nutrient utilization strategy affect the growth rate of microbial cells, microorganisms can grow on a variety of growth substrates and hence possess versatile metabolic activities, previous literature reported that carbon sources are categorized into groups based on their mechanism of joining the metabolic pathway [48], in our study higher population scores were recorded on using glucose/pyruvate, these results are consistent with those of Wang et al [50] who reported that the bacterial isolates tend to co-utilize both sources simultaneously leading to catabolite motivation. It is well known that the efficient applicability of MFC systems depends on the enzymatic degradation ability of the electrogenic microorganisms, to overcome this challenge mixed cultures were suggested [51], so using the two most potent electrogenic bacterial isolates (*E. cloacae* and *E. coli*) in consortium was significantly more efficient than using them individually. In agreement with the findings of Khater et al [22] who reported that, regular addition of fresh substrate overcome the depletion of nutrients and activate

the bacterial culture leading to the restoration of the cell potential, feeding the MFC with 100mL glucose/pyruvate at regular intervals maintain a steady energy generation along the interval of operation. Trying to improve the performance of MFCs, the bacterial consortium was subjected to LLRL to enhance the cell proliferation, protein synthesis and metabolic activities [20]. Previous studies on the effect of laser radiation indicates its bio-stimulatory effect, this effect returned to the photoexcitation of the protein cytochrome C complex, consequently it pumps more photons thus the amount of ATP increases leading to increase in the growth rate, on the other hand high irradiation doses causes cytotoxic effect leading to the damage of the cell structure [28], our results are harmonious with those of Crugeira et al [18] who reported that the stimulatory effect of LLRL is dose dependent and has positive influence on thermophilic bacterial kinetics. In an application of assessing the efficiency of new MFC designed using sludge which contain the metabolically active bacterial cells as culture medium was done, addition of exogenous bacterial inoculum of the photo-stimulated bacterial consortium led to significantly high synergistic action that increased the cell performance, as acceleration of cell proliferation consequently led to intensifying the electron flow, also the addition of fresh medium containing glucose /pyruvate (1%) at regular intervals over comes the depletion of nutrients and led to the revival of the endogenous and exogenous microbial flora, thus the mean value of the potential difference was maintained during the time of operation.

## Conclusion

The main objective of this work was to introduce an ecofriendly and economically viable method to generate green electricity, promising results were obtained by utilization of the potent electrogenic bacterial strains *E. coli* and *E. cloacae* as consortium at optimum conditions. Low level red laser was applied to boost the electrogenic potentiality of the bacterial consortium, results declared significant increase in the efficiency of MFC inoculated with the photo-stimulated consortium. The approach of using photo-stimulated bacterial strains serves as a dual solution for both pollution treatment and renewable energy generation. In future work, in depth study of the enzymatic mechanism of the dual role of photo-stimulated electrogenic bacterial consortium in renewable energy and biohydrogen production will be performed.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12934-023-02187-y.

> Supplementary Material 1

### Declarations

### References
1. Lu N, Zhou S, Zhuang L, Zhang J, Ni J. Electricity generation from starch processing wastewater using microbial fuel cell technology. Biochem Eng J. 2009;43:246–51.
2. Cao Y, Mu H, Liu W, Zhang R, Guo J, Xian M, Liu H. Electricigens in the anode of microbial fuel cells: Pure cultures versus mixed communities 06 Biological Sciences 0605 Microbiology. Microb Cell Fact. 2019;18:1–14.
3. Wang J, Deng H, Wu SS, Deng YC, Liu L, Han C, Jiang YB, Zhong WH. Assessment of abundance and diversity of exo-electrogenic bacteria in soil under different land use types. CATENA. 2019;172:572–80.
4. Jatoi AS, Akhter F, Mazari SA, Sabzoi N, Aziz S, Soomro SA, Mubarak NM, Baloch H, Memon AQ, Ahmed S. Advanced microbial fuel cell for wastewater treatment-a review. Environ Sci Pollut Res. 2021;28:5005–19. https://doi.org/10.1007/s11356-020-11691-2.
5. Chakraborty I, Bhowmick GD, Nath D, et al. Removal of sodium dodecyl sulphate from wastewater and its effect on anodic biofilm and performance of microbial fuel cell. Int Biodeterior Biodegrad. 2021;156:105.
6. Enamala MK, Dixit R, Tangellapally A, Singh M, Dinakarrao SMP, Chavali M, Chandrasekhar K. Photosynthetic microorganisms (algae) mediated bioelectricity generation in microbial fuel cell. Concise Rev Environ Technol Innov. 2020;19., Article 100959, https://doi.org/10.1016/j.eti.2020.100959.
7. Ye Y, Ngo HH, Guo W, Chang SW, Nguyen DD, Liu Y, jie Ni B, Zhang X. Microbial fuel cell for nutrient recovery and electricity generation from municipal wastewater under different ammonium concentrations Bioresour. Technol. 2019; 292, Article 121992, 10.1016/J. BIORTECH.2019.121992.
8. Butti SK, Velvizhi G, Sulonen MLK, Haavisto JM, Koroglu EO, Cetinkaya AY, Singh S, Arya D, Modestr JA, Krishn KV. Microbial electrochemical technologies with the perspective of harnessing bioenergy: Maneuvering towardsup-scaling. Renew Sustain Energy Rev. 2016;53:462–76.
9. Rahimnejad M, Adhami A, Soheil Darvari S, Zirepour A, Oh S. Microbial fuel cell as new technology for bioelectricity generation: A review. Alexandria Eng J. 2015;54:745–56.

10. Tharali AD, Sain N, Osborne WJ. Microbial fuel cells in bioelectricity production Front. Life Sci. 2016;9:252–66. https://doi.org/10.1080/21553769.2016.1230787.

11. Shehab NA, OrtizMedina JF, Katuri KP, Hari AR, Amy G, Loga BE, Saikaly PE. Enrichment of extremophilic exoelectrogens in microbial electrolysis cells using Red Sea brine pools as inocula. Bioresour Technol. 2017;239:82–6. https://doi.org/10.1016/j.biortech.2017.04.122.

12. Munoz-Cupa C, Hu Y, Xu CC, Bassi A. An overview of microbial fuel cell usage in wastewater treatment, resource recovery and energy production. Sci Total Environ. 2020;754:142429.

13. Xiao L, Yaobin L, Haiping L, et al. Effect of pH on bacterial distributions within cathodic biofilm of the microbial fuel cell with maltodextrin as the substrate. Chemosphere. 2021;265:129088.

14. Zha Z, Zhang Z, Xiang P, et al. Porous graphitic carbon from mangosteen peel as efficient electrocatalyst in microbial fuel cells. J Sci Total Environ. 2021;764:142918.

15. Chen S, Patil SA, Brown RK, et al. Strategies for optimizing the power output of microbial fuel cells: Transitioning from fundamental studies to practical implementation. Appl Energy. 2019;233/234:15–28.

16. Prado A, Berenguer R, Esteve-Núñez A. Electroactive biochar outperforms highly conductive carbon materials for biodegrading pollutants by enhancing microbial extracellular electron transfer. Carbon. 2019;146:597.

17. Kim C, Wilkins K, Bowers M, Wynn C, Ndegwa E. Influence of PH and Temperature on Growth Characteristics of Leading Foodborne Pathogens in a Laboratory Medium and Select Food Beverages. Austin Food Sci. 2018;3(1):1031.

18. Crugeira PJ, Santos GM, deOliveira S, Sampaio I, Ferreira E, Chinalia F, Pinheiro A, deAlmeida P. Effect of photostimulation on the catabolic processes of xenobiotics. JPPA: Biology. 2019;191:38–43.

19. Singh SC, Zeng H, Guo C, Cai W, Lasers. Fundamentals, Types, and Operations. Nanomaterials: Processing and Characterization with Lasers; Wiley-VCH Verlag & Co. KGaA, 2012;1–34.

20. Chunga W, Petrofskyb JS, Laymonc M, Logolusoa J, Parka J, Leea J, Leeb H. The effects of low-level laser radiation on bacterial growth. Phys Ther Rehabil Sci. 2014;3:20–6.

21. Bhuyana S, Yadava M, Giria SJ, Sarkara S, Beguma S, Jayswala A, Kabyashreea K, Kumara A, Mandala M, Raya SK. Micro-colony observation: A rapid and simple approach to count bacterial colony forming units. bioRxiv preprint. 2022. https://doi.org/10.1101/2022.01.26.477842.

22. Khater D, El-khatib KM, Hazaa M, Hassan RYA. Activated Sludge-based Microbial Fuel Cell for Bioelectricity Generation. J Basic Environ Sci. 2015;63–73.

23. Hemdan BAE, El–Taweel G, Naha S, Goswami P. Bacterial community structure of electrogenic biofilm developed on modified graphite anode in microbial fuel cell. Sci Rep. 2023;13:1255.

24. Henning C, Gautam D, Muriana P. Identification of multiple bacteriocins in *Enterococcus* spp. using an *Enterococcus* specific bacteriocin PCR array. Microorganisms. 2015;3:1–16.

25. Ibrahim J, Eisen JA, Jospin G, Coil DA, Khazen G, Tokajian S. Genome Analysis of *Streptococcus pyogenes* Associated with Pharyngitis and Skin Infections. PLoS ONE. 2016;11:12e0168177.

26. Naureen Z, Al Matani ZA, Al Jabri MN, Al Housni SK, Gilani SA, Mabood F, Farooq S, Hussain J, Al Harrasi A. Generation of Electricity by Electrogenic Bacteria in a Microbial Fuel Cell Powered by Wastewater. Adv Biosci Biotech. 2016;7:329–35.

27. Saha TC, Protity AT, Zohora FT, Shaha M, Ahmed I. Microbial Fuel Cell (MFC) Application for Generation of Electricity from Dump- ing Rubbish and Identification of Potential Electrogenic Bacteria. Adv Ind Bio- technol. 2019;2:1–8.

28. Ezzat SM, Ahmed NA. Short Short-Term Biodegradation of Crude Petroleum Oil in Water by Photostimulated *Janibacter terrae* Strain S1N1. ACS Omega. 2022;7:13976–84.

29. UN Department of Economic and Social Affairs (UNDESA), World Population Prospects 2019–Highlights; UN: New York, NY, USA., 2022; Availableonline: https://population.un.org/wpp/Publications/Files/WPP2019_Highlights.pdf.

30. Lin H, Ma X. Simulation of co-incineration of sewage sludge with municipal solid waste in a grate furnace incinerator. Waste Manag. 2012;32:561–7.

31. Brahimi R, Cheur W, Laidoudi M, Aouati MK, Bougherara H, Kebabi B. Physical, chemical and spectroscopic analysis of sludge from sewage treatment plant of Mila Algeria for its valorization. Institute of Chemistry, Slovak Academy of Sciences.2021. https://doi.org/10.1007/s11696-021-01814-5.

32. Mucha Z, Kułakows P. Turbidity measurements as a tool of monitoring and control of the SBR effluent at the small wastewater treatment plant-preliminary study. Arch Environ Prot. 2016;42:3:33–6.

33. Xu X, Liu W, Tian S, Wang W, Qi Q, Jiang P, et al. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: a perspective analysis. Front Microbiol. 2018;9:2885.

34. Ni J, Shi H, Xu Y, Wang QA. Comparison of the mechanism of TOC and COD degradation in rhodamine B wastewater by a recycling-flow two- and three-dimensional electro-reactor system. Water. 2020;12:1853.

35. Madni FB, Mutwali EM, Selman HM. Physicochemical and microbiological assessment of drinking water quality in Swakin city. GSC biol pharm sci. 2022;18:3:108–12.

36. Tatinclaux M, Gregoire K, Leininger A, Biffinger JC, Tender L, Ramirez M, Kjellerup BV. Electricity generation from wastewater using a floating air cathode microbial fuel cell. Water-Energy Nexus. 2018;1,2:97–103. https://doi.org/10.1016/j.wen.2018.09.001.

37. Bose D, Kandpal V, Dhawan H, Vijay P, Gopinath M. Energy recovery with microbial fuel cells: bioremediation and bioelectricity in Waste Bioremediation, ed. by Sunita V. Springer, Gujarat, India. 2018; 7–33.

38. Chabert N, Amin AO, Achouak W. All ecosystems potentially host electrogenic bacteria. Bioelectrochemistry. 2015;106:88–96.

39. Yuan SJ, Li WW, Cheng YY, He H, Chen JJ, Tong ZH, Lin ZQ, Zhang F, Sheng GP, Yu HQ. A plate-based electrochromic approach for the high-throughput detection of electrochemically active bacteria. Nat Protoc. 2014;9:112–9.

40. Puvanendran V, Rud I, Breiland MSW, Arnesen JA, Axelsson L. Probiotic *Carnobacterium divergens* increase growth parameters and disease resistance in farmed Atlantic cod (*Gadus morhua*) larvae without influencing the microbiota. Aquaculture. 2021;532:736072.

41. Vasileiou NGC, Chatzopoulos DC, Cripps PJ, Ioannidi KS, Gougoulis DA, Chouzouris TM, Lianou DT, Calvo Gonzalez-Valerio T, Guix Vallverdu R, Argyros S, et al. Evaluation of efficacy of a biofilm-embedded bacteria-based vaccine against *Staphylococcal mastitis* in sheep-A randomized, placebo-controlled field study. J Dairy Sci. 2019;102:9328–44.

42. Ryan MP, Sevjahova L, Gorman R, White S. The Emergence of the Genus *Comamonas* as Important Opportunistic Pathogens. Pathogens. 2022;11:1032.

43. Nguyena DT, Tamuraa T, Tobeb R, Miharab H, Taguchia K. Microbial fuel cell performance improvement based on FliC-deficient *E. coli* strain. Energy Rep. 2020;6:763–7.

44. Matsumoto T, Sugano M. 16S rRNA gene sequence analysis for bacterial identification in the clinical laboratory. Rinsho Byori. 2013;61(12):1107–15.

45. Peyraud R, Kiefer P, Christen P, Portais JC, Vorholt JA. Coconsumption of methanol and succinate by *Methylobacterium extorquens* AM1. PLoS ONE. 2012; 7, e48271.

46. Escalante-Chong R, et al. Galactose metabolic genes in yeast respond to a ratio of galactose and glucose. Proc Natl Acad Sci USA. 2015;112:1636–41.

47. Azmi NM, Anandita N, Tajarudin HA, Shoparwe NF, Makhtar MMZ. Membrane-less microbial fuel cell: effect of pH on the electricity generation powered by municipal food waste. J Phys : Conf Ser. 2021;2129:012095.

48. Callaghan AV. Enzymes involved in the anaerobic oxidation of n-alkanes: from methane to long chain paraffins. Front Microbiol. 2013;4:89–97.

49. Sihag S, Pathak H, Jaroli D. Factors affecting the rate of biodegradation of polyaromatic hydrocarbons. Int J Pure App Biosci. 2014;2:185–202.

50. Wang X, Xia K, Yang X, Tang C. Growth strategy of microbes on mixed carbon sources. Nat Commun. 2019;10:1279.

51. Savvidou MG, Pandis PK, Mamma D, Sourkouni G, Argirusis C. Organic Waste Substrates for Bioenergy Production via Microbial Fuel Cells: A Key Point Review. Energies. 2022;15:5616.

## Publisher's Note