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Full Length Research Article

Bacillus sp. BVB01AS ELECTROGENIN THE TWO CHAMBER MICROBIAL FUEL CELL

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ABSTRACT

The various parameters were studied for the MFC inoculated with nano-wire forming electrogen *Bacillus sp.* BVB01to check their effect on the bioelectricity generation. It was observed that mineral media M6 produced highest bioelectricity of 1461.18mW/m^2 (470.5mV, 0.005mA and 3.1mA/m^2) on the 6th day of the MFC operation at highest external resistance of 100K. Also, it was recorded that on the optimization of various factors, a significant bioelectricity generation of 373739.13mW/m^2 (245.6mV, 2.45mA and 1521.73mA/m^2) was observed from the electrogen *Bacillus* sp. BVB01 on the 26th day at 100Ω was recorded. It was inferred that the bioelectricity production was directly proportional to the biofilm and biomass accumulated at the anode and anolyte of the MFCs.

INTRODUCTION

Microbial Fuel Cells (MFCs) are electrochemical devices that convert chemical energy into electrical energy through the catalytic activity of microorganisms (Allen and Bennetto 1993). Bacteria which are useful for the MFC operation have the ability to transfer electrons to an electrode (anode), as terminal electron acceptors are classified as Electrochemically Active Bacteria (EAB) or Electrogenic Bacteria (Rabaey *et al*., 2004). Electrons are conducted through external circuits to the cathode where they combine with oxygen and protons from anode through the Proton Exchange Membrane (PEM) to form water. Different genetic groups of bacteria have shown exoelectrogenic activity in MFCs (Bond and Lovley, 2003). It has been demonstrated that cell-bound outer membrane cytochromes and conductive pili (nano-wires) may play a key role in electron transfer for some exoelectrogenic bacteria like *Geobacter* and *Shewanella* species (Myers and Myers, 1992; Reguera *et al*., 2005, Gorby *et al*., 2006,). Alternatively, some exoelectrogens, such as *Pseudomonas aeruginosa* (Rabaey *et al*., 2004) and *Geothrixfermentans* (Bond and Lovley, 2005), excrete mediators to shuttle electrons to anode surfaces.

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Also, it was reported that the MFCs should be optimized in terms of reactor configuration and electrolyte to reduce the internal resistance and enable operation to the full microbial catalytic potential (Liu *et al*., 2005). Although, some of the nano-wire forming electrogens like *Geobacter* sp. and *Shewanella* sp. has been studied in the MFC for the electricity production but the effect of physiochemical parameters and effect of two chambers MFC configuration on the bioelectricity generation from nano-wire forming electrogen from genus *Bacillus* has not been reported till date. Hence, the in present work the nano-wire forming electrogen *Bacillus sp*. BVB01culture was inoculated in the two chambers MFC with PEM and the effect of various factors for the bioelectricity generation from the respective MFC was studied.

MATERIALS AND METHODS

Electrogenic Bacteria

The potential electrogen *Bacillus* sp. BVB01was isolated and identified using the 16S rDNA technique in the initial phase of the experiment from the anodic biofilms of MFC inoculated with sea sediment sample (Shiv and Gireesh, 2013) and stored in the bacterial culture collection of Bio Genics Lab, India for the following study.

Dual-Chambered MFC Setup

The dual-chambered MFC was fabricated using PVC bottles (100mL capacity) provided with wire inputs from the top with inlet and outlet ports. The anode and cathode chambers were detached with the aid of cellophane membrane (0.35nm pore size) procured from Himedia, Mumbai, India used as PEM for the MFC. The graphite pencils (Apollo pencil manufacturers, Mumbai, India) were employed as respective electrodes (specific surface area of $0.0014m^2$) and placed at a distance of 3cm on either side of the PEM. The electrodes were injected into respective chambers after aiding with initial wetting (Allen and Bennetto, 1993) followed by circuit connections with the copper wires fixed into the drilled holes of electrodes and sealed with epoxy resin to avoid corrosion of copper wire (Zou *et al*., 2007). The MFC setups were also sterilized with 70% ethanol and dried under UV light for 20min. The selected electrogen *Bacillus* sp. BVB01 was grown overnight in the standard LB media, inoculated with 1% inoculum, prior to inoculation (25%, v/v) in the 75mL mineral media as anolyte for the anodic compartments while the 100mM phosphate buffer enriched with 100mM of potassium hexacyanoferrate was used as catholyte (Kassongo and Togo, 2010). Initially, MFCs were operated for ten days on the closed circuit (100KΩ as external resistance) for all the parameter study and later for one month after the optimization while polarization curves on external resistance were drawn for the Power Density (PD) (mW/m^2) and Current Density (CD) (mA/m^2) normalized to the projected surface area of the anode where power generation was calculated using the equation; P = VI by measuring the voltage (V) and current (I) after an observation of 15min following every 24h for bioelectrochemical analysis, using the digital multimeter (UNI-DT830D, Uni-Trend Group Ltd., Kowloon, Hong Kong).

Screening of different growth media as anolyte

The nano-wire forming electrogen strain BVB01 was inoculated (1%) in 25mL of different mineral media were (g/L): M1 (KH_2PO_4, 1.5; $K_2HPO_4.3H_2O$, 2.9; $(NH_4)_2.SO_4$, 1.3; $MgCl_2.6H_2O$, 0.1; $CaCl_2$, 0.02; Yeast extract, 5.0; $FeSO_4$ solution, 5%; Resazurine, 0.2%; Cysteine hydrochloride, 0.5 and NaCMC, 0.1); M2 ($CaCl_2.2H_2O$, 0.04; $MgSO_4.7H_2O$, 0.1; $NaHCO_3.H_2O$, 1.8; KH_2PO_4, 0.42; NH_4Cl, 0.22; KCl, 0.38; Vitamin/Mineral, 10mL; CH_3COONa, 10mM; Fumarate, 40mM and Cysteine, 1mM); M3 (NH_4Cl, 1.05, KCl, 0.1; $NaH_2PO_4.H_2O$, 4.90; Na_2HPO_4, 9.15; Yeast extract, 0.2; Vitamins/Minerals, 10mL and NaCMC, 1.0); M4 (NH_4Cl, 1.05, KH_2PO_4, 1.5; K_2HPO_4, 2.9; Yeast extract, 0.2; $NaOOCH_3$, 10mM and Vitamin/Minerals, 10mM); M5 (K_2HPO4, 0.13; $MgCl_2$, 4.0; $ZnCl_2$, 0.034; Vitamin, 7.5mL; Starch, 10.0; Beef extract, 10.0; DL-alanine, 10.0; Glycerol, 20.0mL and Seawater, 10%) and M6 (Tryptone, 10.0; NaCl, 10.0; Yeast extract, 5.0 and Sodium acetate, 10.0) served as anolytes in the fabricated two chambers MFC. The 25mL of 24h old bacterial culture in all the types of mineral media were poured into the anodic chambers of MFCs containing respective 75mL sterilized media as anolyte while 100mM potassium hexacyanoferrate enriched in 100mM phosphate buffer as catholyte (Kassongo and Togo, 2010). The electricity generation was then monitored from the MFC setups and media which produced maximum bioelectricity was taken as standard media for further study.

Optimization of standard anolyte

The standard media selected as standard anolyte for the MFC setup was further optimized against 100mM potassium hexacyanoferrate as catholyte in order to assess maximum bioelectricity production from the nano-wire forming electrogenic strain BVB01 by analyzing the following parameters.

Addition of carbon and nitrogen supplements

Five 1% different carbon sources (NaCMC, lactose, glucose, starch and sucrose) and 1% different nitrogen sources (ammonium acetate, urea, ammonium chloride, ammonium sulphate and potassium nitrate) were added separately in the selected anolyte to study their respective effect on the bioelectricity production from the electrogenic strain BVB01 inoculated in the MFCs.

Optimization of catholyte

The 100mL of standard catholyte was also manipulated by using different concentrations (25, 50, 75, 100 and 125mM) of potassium hexacyanoferrate enriched in 100mM phosphate buffer in the cathodic chambers against the standard anolyte M6 in the fabricated MFC setup to check their effect on the net bioelectricity generation.

Optimization of distance between the electrodes

In the constructed MFC setups, the effect of distance (1.5, 3 and 5cm) between the electrodes placed in the respective chambers containing 100mL of standard anolyte and standard catholyte was also monitored for the bioelectricity generation. The distance between the electrodes was varied by changing the length of clamps holding the PEM while keeping PEM at the locus point of the electrodes.

Optimization of surface area of PEM

The different surface areas of the PEM were also checked for the bioelectricity generation from the fabricated MFC setup. The two circular pieces of PEM of radii (1 and 2cm) were fixed in the clamps attached to the chambers of the MFC filled with standard anolyte and catholyte were also analyzed for the bioelectricity generation.

Optimization of electrode surface area

The graphite electrodes used in the MFCs were varied in their superficial surface area to observe their effect on the bioelectricity generation. The MFC setups were constructed keeping the surface areas of the both the electrodes $0.0014m^2$; anode surface area of $0.0028m^2$ and cathode surface area of $0.0014m^2$ as well as anode surface area of $0.0014m^2$ and cathode surface area of $0.0028m^2$in combination for the MFC setups for the bioelectricity generation.

Optimization of external resistance

The five new MFC setups were fabricated and bioelectricity generation was recorded on the external resistances of 10Ω, 100Ω, 1K, 10KΩ and 100KΩ in the circuit. The external

resistance value at which maximum bioelectricity generation was recorded was then opted as the optimized external resistance for the fabricated MFC.

Analysis of bioelectricity generation from optimized MFC

Following optimization of different parameters essential for the enhancement of bioelectricity generation from the MFC with *Bacillus sp*. BVB01, the optimized MFC setup was fabricated and operated for one month while 75% anolytes and 100% catholytes were replaced from the anodic and cathodic chamber, respectively following every ten days interval.

Other analysis

Biomass measurement

Biomass (protein content) attached to the electrode was determined according to Bradford (1976) using Bovine Serum Albumin (BSA) as standard obtained from the Aldrich Sigma (Mumbai, India). Immediately after one month of electrochemical analysis with the optimized parameters for the MFC setup, the respective anode was taken out of the chamber and dipped in the growth medium to remove planktonic cells. It was further incubated in 1mL of 0.2M NaOH at 96°C for 20min to remove the attached biomass. This solution served as protein sample. Furthermore, 0.5mL of 1X Bradford reagent [20mg Coomassie Brilliant Blue G-250 dissolved in 10mL of 95% (v/v) alcohol and 20mL of o-phosphoric acid, volume made up to 200mL with distilled water] was mixed with the 0.5mL protein sample and incubated for 5min at room temperature. The color change was monitored for its absorbance at 595nm against Bradford reagent as blank. The absorbance obtained was compared with the standard curve of BSA, prepared by using different concentrations of BSA (0-500µg) mixed with 5mL of Bradford reagent. The biomass obtained was then expressed in mg/mL.

Biofilm formation assay

In order to examine the biofilm formation, crystal violet (CV) biofilm assays were performed using a modification of a previously described protocol (O'Toole *et al*., 1999). The electrogen *Bacillus*sp. BVBO1 was inoculated (1%) in the 300µL optimized media, further incubated at 35°C for 72h in the 92 well micro-titre plate. Following incubation, the culture was pipette out gently and 300µL of 0.01% (w/v) CV solution was added to stain the bacterial cells attached to the walls of plates. It was allowed to incubate for 15min before wells were rinsed to remove unattached cells. Remaining cells were dried for 20min at room temperature and then the CV solution was solubilized with 300µL of 100% dimethyl sulfoxide. The OD_{600} was taken to monitor the cell attachment on the walls of well using spectrophotometer.

RESULTS AND DISCUSSION

Screening of anolytes

The different mineral media served as anolytes in the MFC anodic chambers were inoculated with the *Bacillus* sp. BVB01 and bioelectricity generations wererecorded. It was observed that the mineral media, M1 showed maximum bioelectricity generation of 282.36mW/m^2 (227.3mV, 0.002mA and 1.24mA/m^2) on the 5th day (Fig.1A), M2 showed maximum bioelectricity generation of 705.65mW/m^2 (378.7mV, 0.003mA and 1.86mA/m^2) on the 4th day (Fig.1B), M3 represented highest bioelectricity generation of 225.21mW/m^2 (181.30mV, 0.002mA and 0.124mA/m^2) on the 4th day (Fig.1C), M4 depicted maximum electricity generation of 977.39mW/m^2 (393.4mV, 0.004mA and 2.48mA/m^2) on the 4th day (Fig.1D), M5 reflected significant bioelectricity production of 945.34mW/m^2 (380.5mV, 0.004mA and 2.48mA/m^2) on the 3rd day (Fig.1E) and M6 presented maximum bioelectricity generation of 1461.18mW/m^2 (470.5mV, 0.005mA and 3.1mA/m^2) on the 6th day (Fig.1F) from the MFC setup.

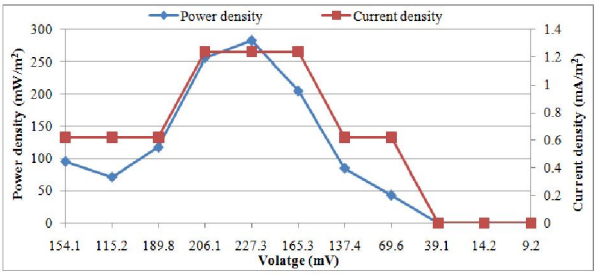

Fig.1(A). Screening of bioelectricity generation from *Bacillus* sp. BVB01 with mineral media M1 in MFC

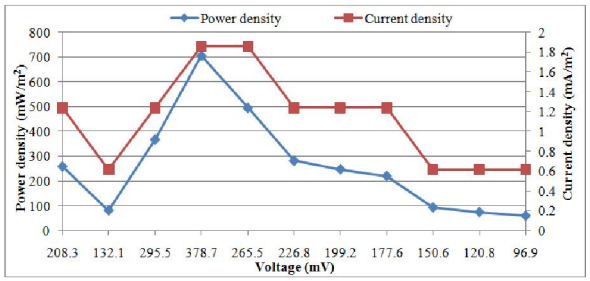

Fig.1(B). Screening of bioelectricity generation from the *Bacillus* sp. BVB01 with mineral media M2 in MFC

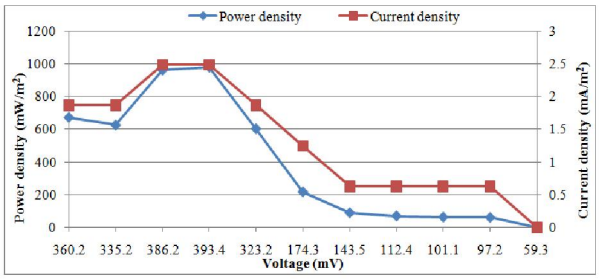

Fig.1(C). Screening of bioelectricity generation from the *Bacillus* sp. BVB01 with mineral media M3 in MFC

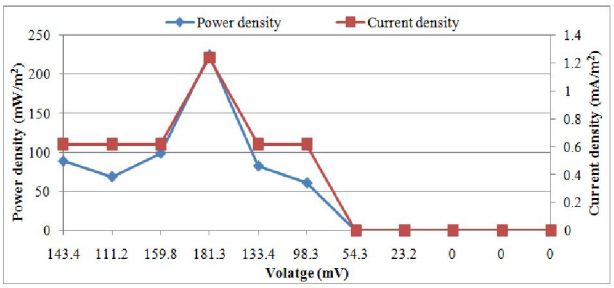

Fig.1(D). Screening of bioelectricity generation from the *Bacillus* sp. BVB01 with mineral media M4 in MFC

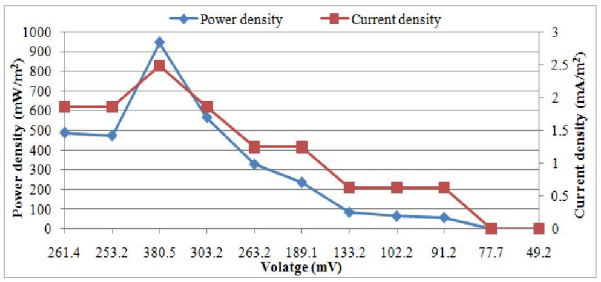

Fig.1(E). Screening of bioelectricity generation from the *Bacillus* sp. BVB01 with mineral media M5 in MFC

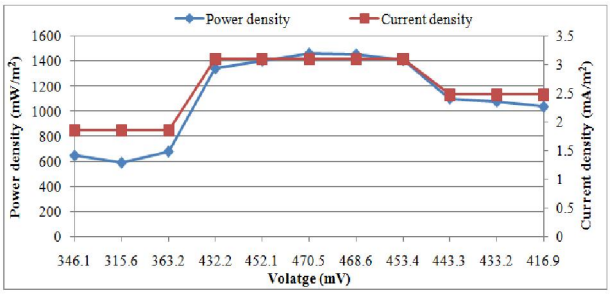

Fig.1(F). Screening of bioelectricity generation from the *Bacillus* sp. BVB01 with mineral media M6 in MFC

Based on the obtained results, it was deduced that mineral media M6 generated maximum bioelectricity and hence, opted as the standard anolyte for the *Bacillus* sp. BVB01.

Analysis of media optimization

The standard mineral media M6 was further optimized in order to evaluate the maximum electric potential of the isolated electrogenic strain BVB01 in the MFC by studying the following different parameters.

Effect of carbon and nitrogen supplements

The bioelectricity generation was monitored from the MFCs with the electrogenic strain BVB01 inoculated in the selected standard mineral media M6 amended with five different carbon sources individually. It was noticed that among the different carbon sources amended into the anolyte, the maximum bioelectricity generation of 1466.14mW/m^2 (472.10mV, 0.005mA and 3.1mA/m^2) showed with CMC on the 6th day (Fig.2A), 2101.49mW/m^2 (563.90 mV, 0.006mA and 3.7mA/m^2) noticed with lactose on the 5th day (Fig.2B), 2333.66mW/m^2 (626.2mV, 0.006mA and 3.72mA/m^2) recorded with glucose on the 8th day (Fig.2C), 2155.24mW/m^2 (589.10mV, 0.006mA and 3.72mA/m^2) observed with starch

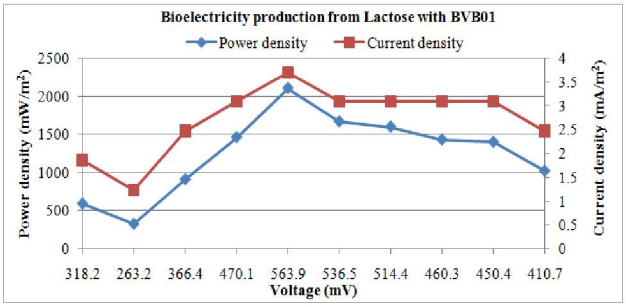

Fig.2(A). Screening of CMC for bioelectricity generation from BVB01

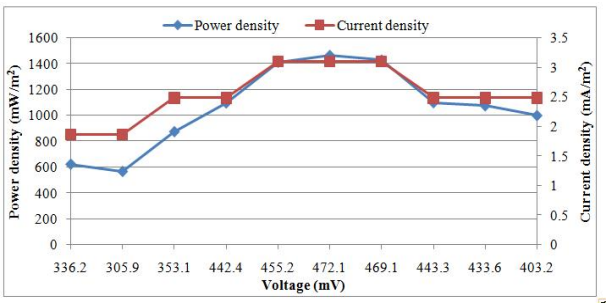

Fig. 2(B). Screening of Lactose for bioelectricity generation from BVB01

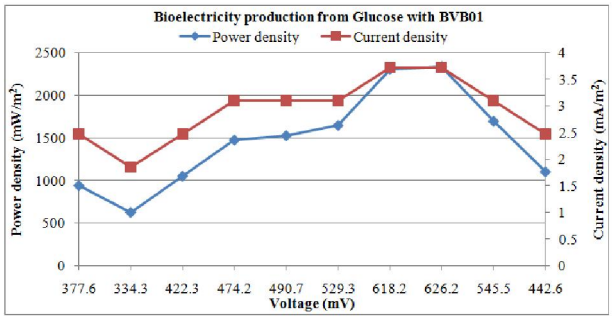

Fig.2(C). Monitoring of Glucose for bioelectricity generation from BVB01

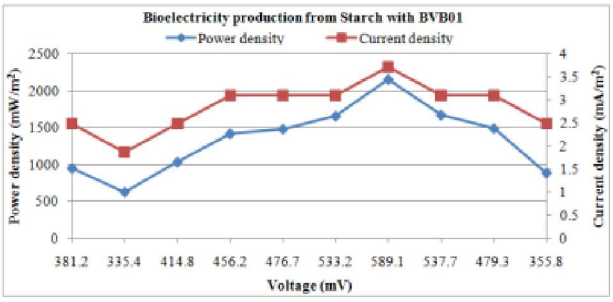

Fig.2(D). Screening of Starch for bioelectricity generation from BVB01

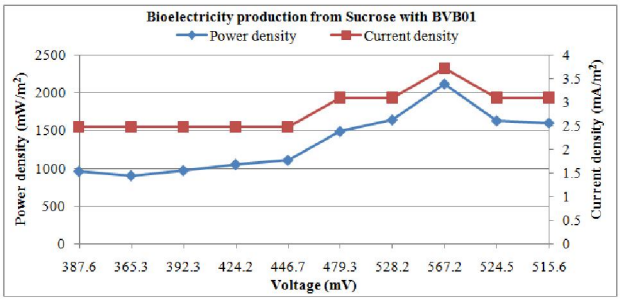

Fig.2(E). Screening of Potassium nitrate for bioelectricity generation from BVB01

on the 7th day (Fig.2D) and 2113.78mW/m^2 (567.20mV, 0.006mA and 3.72mA/m^2) showed with sucrose on the 8th day (Fig. 2E); from the MFC setups. The readings obtained recommended the use of glucose as carbon supplement for the electrogen *Bacillus* sp. BVB01, in the standard mineral medium. Similarly for the five different nitrogen sources amended into the mineral media, it was observed that ammonium acetate yielded maximum bioelectricity of 2101.49mW/m^2 (563.90mV, 0.006mA and 3.72mA/m^2) on the

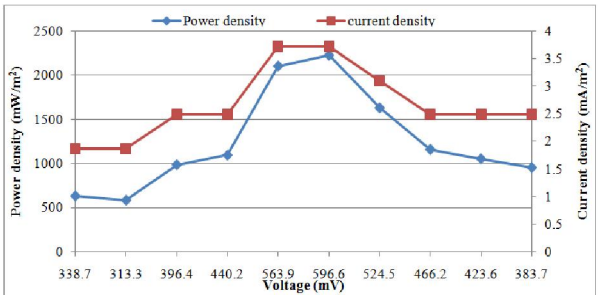

Fig.3(A). Screening of Ammonium acetate for bioelectricity generation from BVB01

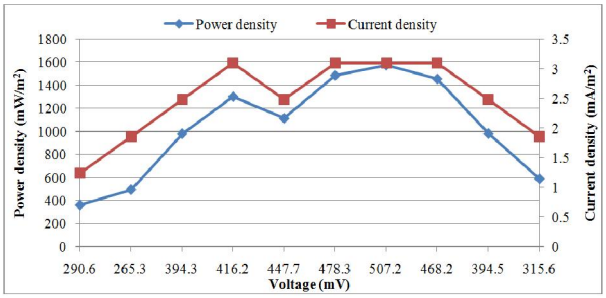

Fig.3(B): Screening of Urea for bioelectricity generation from BVB01

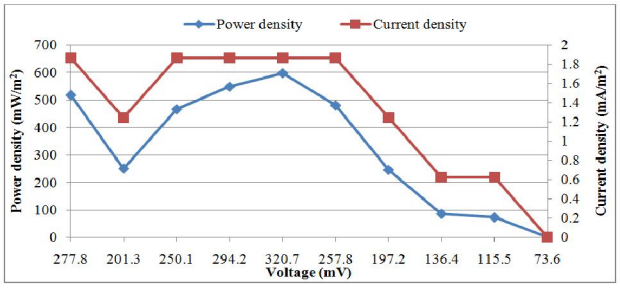

Fig.3(C). Monitoring of Ammonium chloride for bioelectricity generation from BVB01

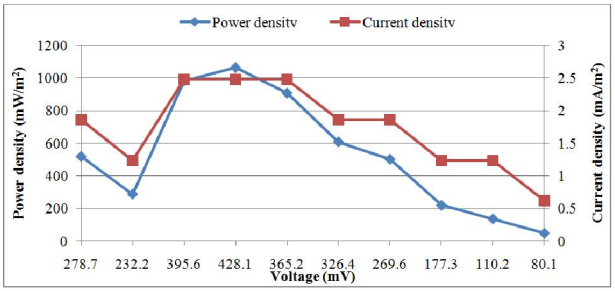

Fig.3(D). Screening of Ammonium sulphate for bioelectricity generation from BVB01

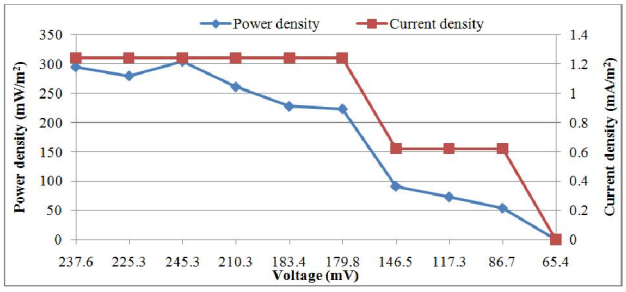

Fig.3(E). Screening of bioelectricity generation from Potassium nitrate using BVB01

5th day (Fig.3A), urea produced maximum bioelectricity of 1575.15mW/m^2 (507.2mV, 0.005mA and 3.1mA/m^2) on the 7th day (Fig.3B), ammonium chloride generated the maximum bioelectricity of 597.57mW/m^2 (320.70mV, 0.003mA and 1.86mA/m^2) on the 6th day (Fig.3C), ammonium sulphate induced maximum bioelectricity generation of 1063.60mW/m^2 (428.10mV, 0.004mA and 2.48mA/m^2) on the 4th day (Fig.3D) and potassium nitrate reflected maximum of 304.72mW/m^2 (245.30mV, 0.002mA and 1.24mA/m^2) on the 3rd day (Fig.3E) of the MFC operation inoculated with *Bacillus* sp. BVB01. Hence, it was concluded that ammonium acetate was an ideal nitrogen source for *Bacillus* sp. BVB01 in the MFC operation for the maximum bioelectricity generation.

Optimization of catholyte

The different concentrations of the potassium hexacyanoferrate enriched in 100mM potassium phosphate buffer (pH 7.2) as catholyte were analyzed for the bioelectricity generation in the cathodic chamber of the MFCs at external resistance of 100K against the anolyte inoculated with the electrogen *Bacillus* sp. BVB01. It was observed that the maximum bioelectricity generation of 256.52mW/m^2 (206.50mV, 0.002mA and 1.24mA/m^2) was taped with 25mM concentration on the 4th day (Fig.4A), 550.62mW/m^2 (295.50mV, 0.003mA and 1.86mA/m^2) was observed with 50mM concentration on the 5th day (Fig.4B), 927.7mW/m^2 (373.40mV, 0.004mA and 2.47mA/m^2) was depicted with 75mM concentration on the 4th day (Fig.4C), 1461.18mW/m^2 (470.50mV, 0.005mA and 3.1mA/m^2) was recorded with 100mM concentration on the 6th day (Fig.4D) and125mM concentration showed 2322.48mW/m^2 (623.20mV, 0.006mA and 3.72mA/m^2) on the 7th day (Fig.4E) of the MFC operation against the anolyte inoculated with *Bacillus* sp. BVB01 in the anodic chamber. It was concluded that with the increase in concentration of potassium hexacyanoferrate the electricity generation also increased. It has been documented that in mediator less microbial fuel cell losses occur in the cathode compartment due to activation over potentials which can be decreased by adding $K_3Fe(CN)_6$ to the liquid catholyte (Park *et al*., 2000).

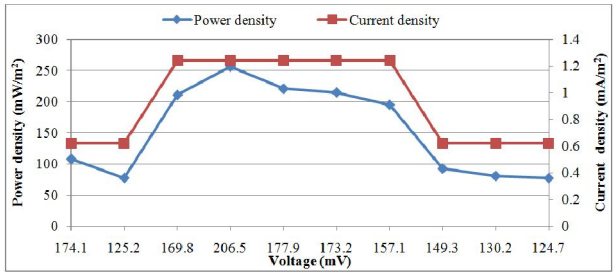

Fig.4(A). Bioelectricity generation from 25mM Potassium hexacynaoferrate as catholyte in the MFC inoculated with BVB01

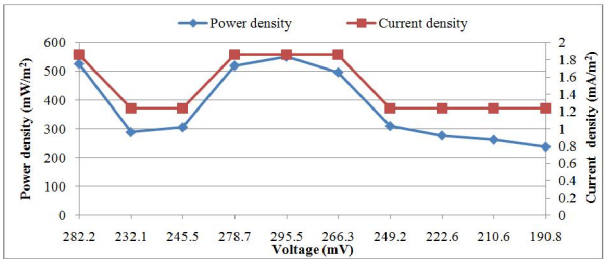

Fig.4(B). Bioelectricity generation using 50mM Potassium hexacynaoferrate as catholyte in MFC inoculated with BVB01

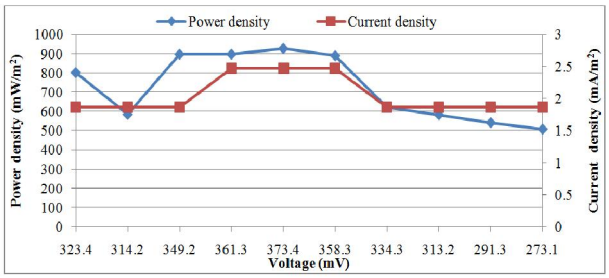

Fig.4(C). Bioelectricity generation from 75mM Potassium hexacynaoferrate as catholyte in the MFC inoculated with BVB01

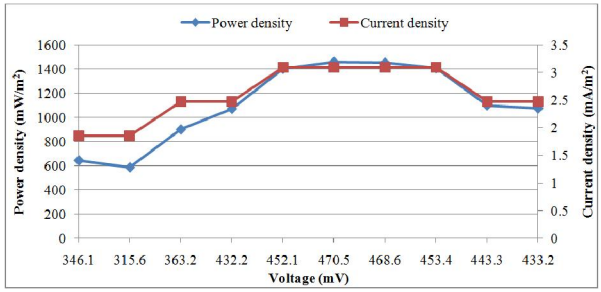

Fig.4(D). Bioelectricity generation from 100mM Potassium hexacynaoferrate as catholyte in the MFC inoculated with BVB01

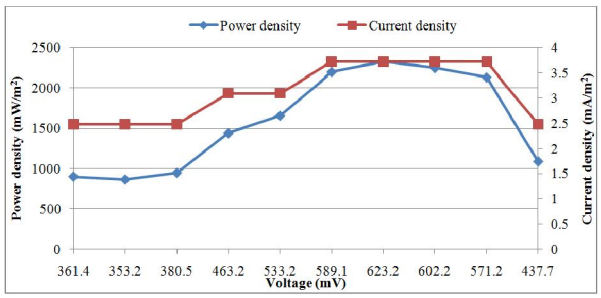

Fig.4(E). Bioelectricity generation from 125mM Potassium hexacynaoferrate as catholyte in the MFC inoculated with BVB01

Effect of distance between electrodes

The bioelectricity generations from the isolated electrogen was also monitored with the change in distance between the electrodes placed in the chambers of the MFC. The maximum bioelectricity generation of 688.50mW/m^2 (369.5mV, 0.003mA and 1.86mA/m^2) on the 6th day with the electrodes having 1.5cm distance (Fig.5A), 1461.18mW/m^2 (470.5mV, 0.005mA and 3.1mA/m^2) on the 6th day with electrodes at 3cm distance (Fig.5B) and 477.57mW/m^2 (256.30mV, 0.003mA and 1.86mA/m^2) on the 6th day with electrodes at 5cm distance (Fig.5C), were recorded from the MFCs inoculated with *Bacillus* sp. BVB01 in the anodic chambers.

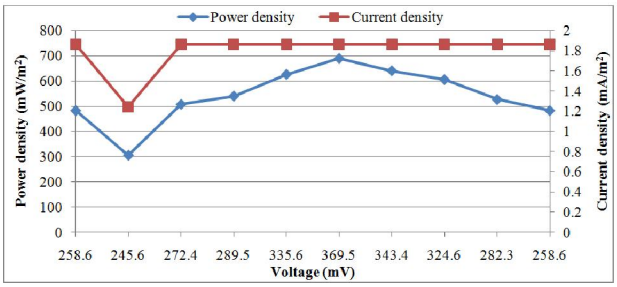

Fig.5(A). Bioelectricity generation from the MFC having electrode separation 1.5cm inoculated with BVB01

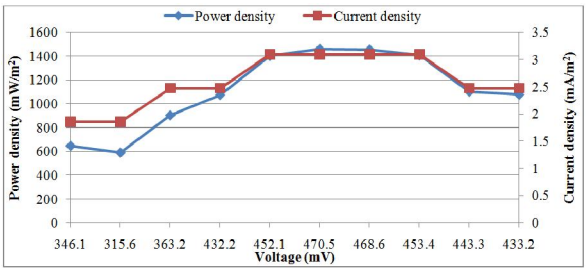

Fig.5(B). Bioelectricity generation from the MFC having electrode separation 3cm inoculated with BVB01

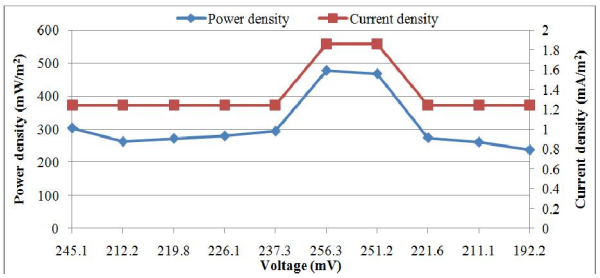

Fig.5(C). Bioelectricity generation from the MFC having electrode separation 5cm inoculated with BVB01

Hence, it was deduced that the 3cm distance between the electrodes used for the MFC was the optimized distance and therefore, employed in further experimental study. It was suggested that the mass transfer between two electrodes is a limiting factor, probably proton transfer from the anode to the cathode. Since, both the anode and the cathode solutions have resistance to proton transfer, therefore optimized distance between the electrodes reduced the resistance of the electrolytes that the protons have to overcome resulted in reduction of microbial fuel cell resistance and higher power output (Yuan and Kim, 2007).

Analysis of surface area of PEM

The surface area of the membrane was also analyzed for the optimization of the MFC setup inoculated with the electrogen *Bacillus* sp. BVB01.

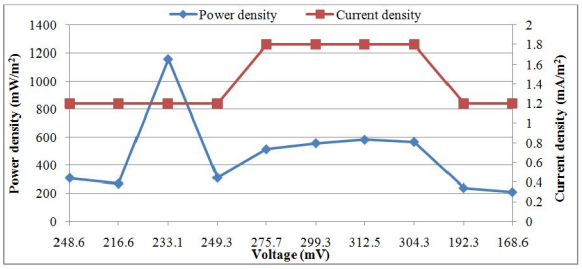

Fig.6(A). Monitoring of bioelectricity generation from the MFC using PEM having 1cm radius inoculated with BVB01

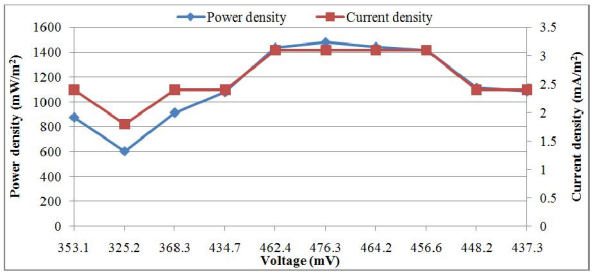

Fig.6(B). Monitoring of bioelectricity generation from the MFC using PEM having 2cm radius inoculated with BVB01

The results obtained revealed the maximum bioelectricity generation of 582.29mW/m^2 (312.5mV, 0.003mA and 1.8mA/m^2) with 1cm radius and 1479.19mW/m^2 (476.30mV, 0.005mA and 3.1mA/m^2) with 2cm radius of circular membrane on the 7th day (Fig.6A) and 6th day (Fig.6B), respectively. From the obtained data, it was concluded that 2cm radius of membrane enabled greater electricity generation, suggesting that membrane with 1cm generated less electricity was probably because of the surface area of the membrane which did not provide enough proton flux through the membrane in the MFC while maximum surface area delivered maximum photons and hence, resulted in maximum electricity generation.

Effect of electrode surface area

In the fabricated MFCs, graphite electrodes were varied in their apparent surface area and their effect on the bioelectricity generation. It was concluded that *Bacillus* sp. BVB01produced maximum bioelectricity generation of 461.18mW/m^2 (470.50mV, 0.005mA and 3.10mA/m^2) on the 6thday with both the electrodes surface area of 16.10cm^2 (Fig.7A), 1893.91mW/m^2 (508.20mV, 0.006mA and 3.72mA/m^2) on the 7th day with anode and cathode surface area of 32.20cm^2 and 16.10cm^2 respectively, (Fig.7B) while 1901.36mW/m^2 (510.20mV, 0.006mA and 3.72mA/m^2) on the 7th day with the anode surface area of 16.32cm^2 and the cathode surface area of 32.10cm^2 (Fig.7C).

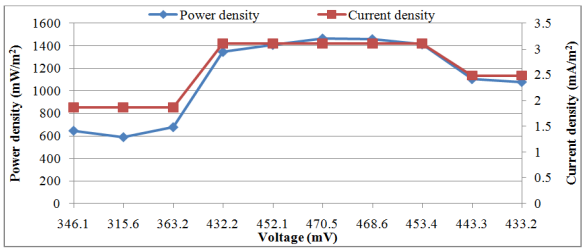

Fig.7(A). Bioelectricity generation using combination of Anode (16.10cm^2) and Cathode (16.10cm^2) from MFC inoculated with BVB01

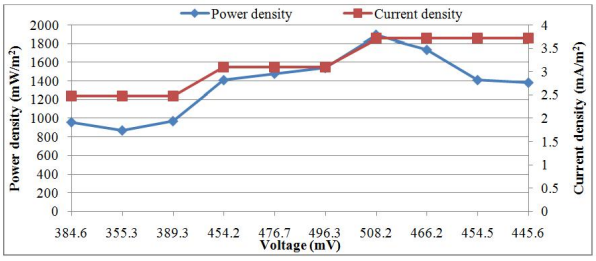

Fig.7(B). Bioelectricity generation using combination of Anode (32.20cm^2) and Cathode (16.10cm^2) from MFC inoculated with BVB01

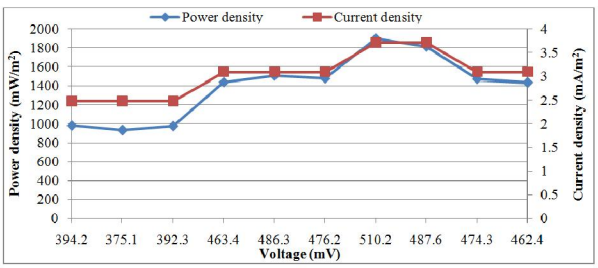

Fig.7(C). Bioelectricity generation using combination of Anode (16.10cm^2) and Cathode (32.20cm^2) from MFC inoculated with BVB01

The results concluded that the combination of 16.10cm^2 and 32.20cm^{2}surface areas of electrodes for the electrogen generated maximum bioelectricity. It was suggested that at the anode more surface area was available to accept the electrons from the bacteria and carry them to the external circuit. The bacteria produced more electrons than the uptake capacity of the cathode and when the surface area of cathode was increased the electrons up take was normalized that reduced the over potential at the anode while at the cathode the extra surface area provided the sufficient oxidant to reduce the incoming electrons from the electrode. This readily facilitated more flow of electrons and thus a larger power output (Cheng *et al*., 2006).

Effect of external resistance

The external resistance on the circuit was also optimized for the system to evaluate maximum bioelectricity generation from the inoculated electrogen. It was noticed that with the change in the external resistance from higher value to lower value, increase in current density production while decrease in power density was recorded. The maximum power density of 197679.5mW/m^2 (178.80mV, 1.78mA and 1105.59mA/m^2) was recorded for *Bacillus* sp. BVB01 at 100Ω resistance (Fig.8). Hence, from the data obtained, it was concluded that for the electrogen inoculated in the MFCs, 100Ω was observed as the optimum external resistance. The obtained results deduced that 100Ω would be equal to or near to the internal resistance of the constructed MFC. It has been reported that the external electric circuit must have a resistance equal to the internal resistance of the microbial fuel cell to produce the maximum amount of power (Kim *et al*., 2008).

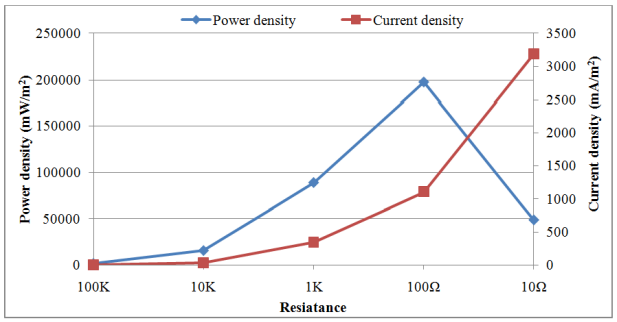

Fig.8. Effect of different external resistance on the bioelectricity generation from MFC inoculated with BVB01

Analysis of bioelectricity generation from optimized MFC

The anodic chamber of MFC setup was inoculated with the *Bacillus* sp. BVB01and bioelectricity production was monitored for one month. It was observed that the maximum bioelectricity production of 373739.13mW/m^2 (245.6mV, 2.45mA and 1521.73mA/m^2) was observed from the *Bacillus* sp. BVB01 on the 26th day (Fig.9) of the MFC operation. Moreover, it was also recorded that the obtained maximum powers from the respective MFCs were constant for almost 1h followed by decrement in the output. It was also noticed that the obtained maximum power from the respective MFC was constant for almost 1h followed by decrement in the output, suggested that the optimized parameters contributed to the reduction of the different resistances and over potentials necessary for the better efficiency of the fabricated MFC setup.

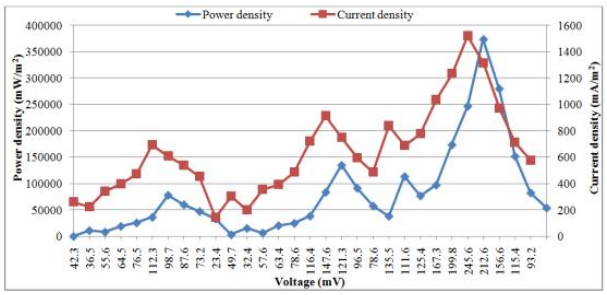

Fig.9. Bioelectricity generation from the MFC under optimized parameters inoculated with *Bacillus* sp.BVB01

Analysis of bacterial biomass and biofilm formation

Following termination of the MFC operation after one month, the electrode and the anolytes were analyzed for the biomass. The anode was employed for estimation of attached protein in form of biofilms whereas the anolyte was analyzed for the biomass productions using Bovine Serum Albumin (BSA) as standard curve (Fig.10). It was noted that *Bacillus* sp. BVB01 showed protein content of 2.3mg/cm^2 and biomass of 5.91mg/mL from the anode and anodic chamber, respectively. .The results for the biofilm attachment assay of the isolated electrogen *Bacillus sp*. BVB01 in the standard media (0.112 λmax) and optimized media (0.178 λmax) revealed that the bacteria showed maximum absorbance in the optimized media as compared tothe standard media, suggested due to happy biofilm formation of the electrogen *Bacillus sp.* BVB01in the optimized media because of its optimized factors necessary for the electrogen biofilm formation.

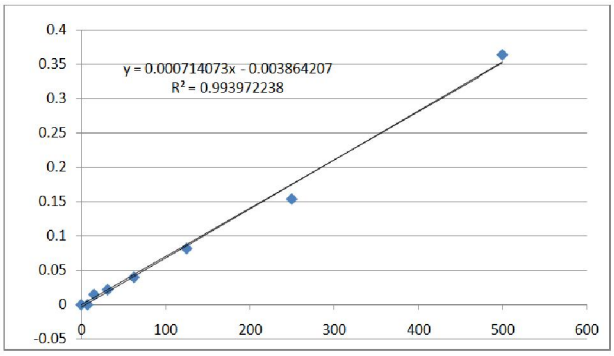

Fig.10. Bovine Serum Albumin (BSA) as standard curve

Conclusion

The present research work emphases on the evaluation of bioelectricity generation from the isolated electrogen *Bacillus sp*.BVB01 from the sea water The electrochemical analysis data obtained revealed that the isolated electrogen have the potential to generate maximum bioelectricity in the optimized MFC. Hence, the potential electrogenic bacteria *Bacillus* sp. BVB01 procured from these a water biofilms and mineral media M6 can be used with necessary requirements for the better usage to understand the MFC technology as alternate energy to sustain the demand of future.

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REFERENCES

Allen, R.M. and Bennetto, H.P. 1993. Microbial Fuel Cells: electricity production from carbohydrates. *Appl. Biochem. Biotech*., 39(40):27- 40.

Bond, D.R. and Lovley, D.R. 2003. Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Appl. Environ. Microbiol*., 69:1548–1555.

Bond, D.R. and Lovley, D.R. 2005.Evidence for involvement of an electron shuttle in electricity generation by *Geothrix fermentans*. *Appl. Environ. Microbiol*., 71:2186–2189.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., 72: 248-254.

Cheng, S., Liu, H. and Logan, B.E. 2006. Increased power generation in a continuous flow MFC with advective flow through the porous anode and reduced electrode spacing, *Environ. Sci. Technol*., 40(7), 2426-32.

Gorby, Y.A., Yanina, Y.S., McLean, J.S., *et al.* 2006. Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *Proc. Natl. Acad. Sci*. USA., 103:11358–11363.

Kassongo J and Togo CA. 2010. The potential of whey in driving microbial fuel cells: A dual prospect of energy recovery and remediation. *Afr, J, Biotechnol*., 9(46):7885-7890.

Kim, S., Chae, K., Choi, M., *et al.* 2008. Microbial Fuel Cell: Recent Advances, Bacterial Communities and application beyond Electricity Generation. *Environ. Eng. Res*., 13(2):52-65.

Liu, H., Cheng, S. and Logan, B.E. 2005. Power generation in fed batch microbial fuel cells as a function of ionic strength, temperature and reactor configuration. *Environ. Sci. Technol*., 39:5488-5493.

Myers, C.R., and Myers, J.M., 1992. Localization of cytochromes to the outer membrane of anaerobically grown *Shewanella putrefaciens* MR-1. *J. Bacteriol*., 174:3429–3438.

O'Toole, G.A, Pratt, L.A, Watnick P.I, *et al.* 1999. Genetic approaches to study of biofilms. *Methods Enzymol*., 310:91-109.

Park, D.H. and Zeikus, J.G. 2000. Electricity generation in microbial fuel cells using neutral red as an electronophore. *Appl. Environ. Microbiol*., 66(4):1292–1297.

Rabaey, K., Boon, N., Siciliano, S.D., *et al.* 2004. Biofuel cells select for microbial consortia that self-mediate electron transfer. Appl. Environ. Microbiol., 70:5373-5382.

Rabaey, K., Boon, N., Siciliano, S.D., *et al.* 2004. Biofuel cells select for microbial consortia that self-mediate electron transfer. *Appl. Environ. Microbiol*., 70:5373–5382.

Reguera, G., McCarthy, K.D., Mehta, T., *et al.*2005. Extracellular electron transfer via microbial nanowires. *Nature*., 435:1098–1101.

Shiv Kumar and Gireesh Babu K. 2013.Isolation and identification of nano-wire forming electrogens from anodic biofilms of the microbial fuel cells (MFCS), *International Journal of Recent Scientific Research*., 4(11):1803-1808.

Yuan, Y. and Kim, S. 2007. Pollypyrrole-Coated Reticulated Carbon as anode in Microbial fuel cell for higher energy output, Bull. *Korean Chem. Soc*., 29(1):168-172.

Zou YJ, Sun LX, XU F and Yang LN. 2007. *E.coli* Microbial Fuel Cell Using New Methylene blue as Electron Mediator. *Chemical Journal of Chinese Universities*, 28(3):510-513.

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