Low-voltage producing microbial fuel cell constructs using biofilm-forming marine bacteria

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Biofilm-forming marine bacterial isolates *Paenibacillus lautus* NE3B01, *Pseudomonas mendocina* NR802, *Stenotrophomonas acidaminiphila* NCW702 and *Pseudomonas pseudoalcaligenes* NP103 in microbial fuel cell (MFC) were investigated for low-voltage power generation. Biofilm formation by the isolates was evaluated by glass tube assay, microtitre plate assay and fluorescence microscopy. A dual chamber MFC of 2 litre capacity was constructed for low-voltage power generation and current output. Two chambers were internally connected by salt bridge and externally the circuit was connected with copper wires which were joined to the electrodes at the two ends and to the multimeter. Maximum current was generated when the salt bridge was constructed using 1 M KCl for all the four bacterial isolates. With *Paenibacillus lautus* NE3B01, a maximum voltage of 727.5 ± 13.4 mV in 6 h with 7 g/l of glucose as the sole source of carbon was recorded. However, *Pseudomonas mendocina* NR802 MFC was the most stable in terms of potential generation among all the isolates used for MFC studies. The experimental data for current and voltage showed that the biofilm-forming marine bacterial isolates are useful in MFC technology.

Keywords: Biofilm, current output, low voltage, marine bacteria, microbial fuel cell.

SINCE long, humans have been dependent on energy sources to fulfil their basic requirements. With increase in energy demand, they started searching for more resources that can be utilized to achieve the energy requirement. In the recent years, microbial fuel cells (MFCs) have emerged as a promising technology to fulfil future energy needs[1]. The depletion of fossil fuels and demand for alternating fuels have paved the way for MFC research. MFC is an ideal way of generating electricity by means of microorganisms. In a MFC, microbes are used to generate electricity from the biochemical energy produced during metabolism of organic substrates[2]. Electricity output can be sustained by microbes as long as organic substrate input is maintained in MFC. Like an electrochemical cell MFC consists of an anode and a cathode chamber, connected by an external circuit and separated by cationic-specific membrane. In the anode chamber, decomposition of organic substrate by the microbes generate electrons (e^-) and protons (H^+) that are transferred to the cathode through the circuit and membrane respectively[3]. Bacteria are the most preferred microbes that can be used in MFCs. Usually a consortium of microbes is used to generate electricity[4,5]. However, some MFCs also explore metabolic tendencies of single bacterial species. For efficient electron transfer to the anode, mediators like dye and metalloorganics are preferred. Conversely, for mediator-less operation, metal-reducing and anodophilic bacteria (e.g. *Geobacter metallireducens*, *Geobacter sulfurreducens*, *Shewanella putrefaciens*, etc.) are preferred[1,6].

The working advantages of MFCs are: (i) use of organic squander stuff as fuels and microbes as catalysts, (ii) they do not require extremely synchronized division systems like those needed for collecting hydrogen gas in hydrogen fuel cells[7], and (iii) they have high substrate conversion rate compared to enzymatic fuel cells as redox enzymes cannot transfer electrons to the conducting surfaces directly[8]. There are many advantages of MFCs over the technologies currently used for generating energy from organic matter[9]. MFC technology confers a reliable transfer of substrate energy to electricity enabling elevated conversion efficiency. MFC can be operated efficiently at variable (optimum and low) temperatures, a distinct feature of this bio-energy process. The gases released from MFC do not require additional treatment[9,10]. The off-gases of MFCs are enriched in carbon dioxide, a gas that has no useful energy content[10]. Optimization of MFC is essentially determined by a bacterial consortium and its adaption to operational conditions. Thus, power output from a MFC is governed by a biological process with the addition of ongoing electrochemical response[9].

The marine environment provides an unexplored pool of many microbes that can be used in mediator-less MFC[11]. Biofilms are multicellular sessile communities of microbes, enclosed in a matrix of self-synthesized extracellular polymeric substances[12]. Biofilm-forming marine bacteria can be a promising target to increase the performance of MFCs, as they can help in electron transport to the electrode[1]. Thus, in the present study, we studied the potential of biofilm-forming marine

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bacteria *Paenibacillus lautus* NE3B01, *Pseudomonas mendocina* NR802, *Stenotrophomonas acidaminiphila* NCW702 and *Pseudomonas pseudoalcaligenes* NP103 for low-voltage power generation in MFC.

Materials and methods

Bacterial strain, media and growth condition

Paenibacillus lautus NE3B01, *Pseudomonas mendocina* NR802, *S. acidaminiphila* NCW702 and *Pseudomonas pseudoalcaligenes* NP103 isolated from Bhitarkanika mangrove ecosystem, Rushikulya estuary, Chilika lagoon and Paradeep port respectively, along Odisha coast, India and identified by 16S rRNA gene sequencing (GenBank accession numbers: JX273779, JX273777, JX514371 and JX273778 respectively) have been used for this study. *Paenibacillus lautus* NE3B01 (MTCC No. 11807), *Pseudomonas mendocina* NR802 (MTCC No. 11808) and *S. acidaminiphila* NCW702 (MTCC No. 11809) have been deposited to Microbial Type Culture Collection and Gene Bank, India. *Pseudomonas pseudoalcaligenes* NP103 (LMG 28190) has been deposited to Belgian Coordinated Collections of Microorganisms, Belgium.

Nutrient agar and broth (Himedia, India), Luria Bertani (LB) broth (Himedia, India) and minimal broth medium [Himedia, India (K_2HPO_4 – 7 g/l, KH_2PO_4 – 2 g/l, sodium citrate – 0.50 g/l, $MgSO_4$ – 0.10 g/l and $(NH_4)_2SO_4$ – 1.0 g/l)] were used in this study. The bacterial isolates were grown at 37°C, 160 rpm in nutrient broth for 12 h. Biofilm experiments were performed in LB broth. For MFC, minimal broth medium was used along with glucose as carbon source at different concentrations.

Biofilm assay and fluorescence microscopic analysis

Screening for biofilm formation was done by borosilicate glass tube assay following the method of O'Toole *et al.*[13] and Jain *et al.*[14], with slight modifications. Briefly, a colony was inoculated in LB broth and incubated at 37°C overnight. Overnight culture was diluted to 1 : 100 in LB broth, and 1 ml was transferred to a fresh glass tube. After 48 h, the tube was washed with distilled water twice, stained with 0.2% crystal violet and destained with 95% ethanol for 30 min. Then 200 µl of destained solution was transferred to a microtitre plate and absorbance at 595 nm was measured to quantify the biofilm growth.

For fluorescence microscopy, isolates were grown on LB broth (37°C, 160 rpm) till log phase. Next 50 µl (1 : 100 dilution) of this culture was inoculated in glass tube containing a glass slide half dipped in 5 ml LB broth. These tubes were incubated for 48 h at 37°C under static condition. After 48 h the glass slides were carefully taken out and washed with autoclaved distilled water, stained with 0.02% acridine orange and air-dried. The slides were then observed under inverted fluorescence microscope (Olympus, 1 × 71, Japan).

MFC components and assembly

Electrode: Graphite felt of dimension 15 × 2 cm was used as cathode and anode for MFC. The electrodes were cleaned with 1% $HgCl_2$ and stored in 0.1 M HCl. Prior to use, the electrodes were soaked in deionized water for a period of 24 h. They were positioned at a distance of 6 cm on either side of a salt bridge (Figure 1)[15].

Salt bridge: The salt bridge was prepared by dissolving 3% agarose in KCl (1 M and 3 M) or NaCl solution (1 M and 2 M). The mixture was boiled for 2 min and casted in PVC pipes (dimension 10 × 3 cm) in aseptic condition. The salt bridge was properly sealed and kept in a refrigerator for proper settling[16].

Microbial fuel cell: Fuel cell was constructed using transparent plastic polyethylene terephthalate bottles. The fuel cell has electrode compartment of approximately 2 litre. Each chamber was provided with sample port, wire point inputs (top), and ports for electrode wire. The two compartments were connected via a salt bridge. The anode compartment was loaded with freshly prepared, autoclaved, minimal broth medium (1.50 litre) with glucose (0.5–10%) as carbon source and 10 ml methylene

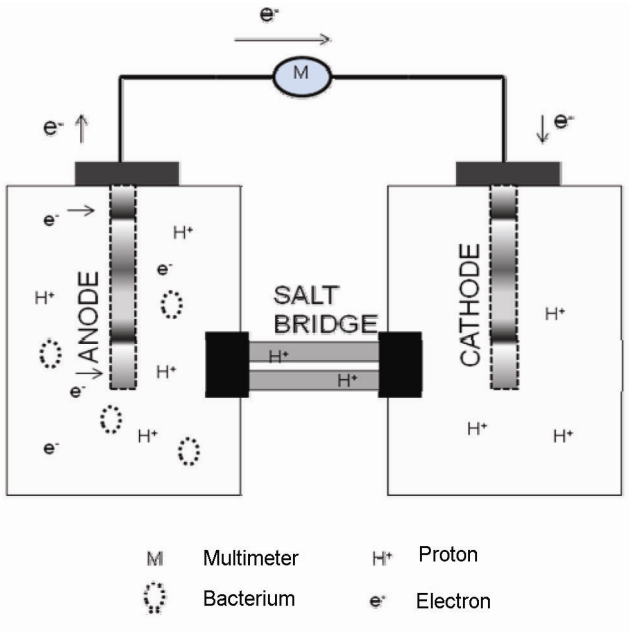

Figure 1. Microbial fuel cell set-up: graphite felt of dimension 15 × 2 cm was used as cathode and anode. The anode compartment was loaded with freshly prepared, autoclaved, minimal broth medium supplemented with glucose (0.5–10%) as carbon source and 10 ml methylene blue as mediator. Cathodic compartment was loaded with 2 litre of 50 mM K_2HPO_4, pH 7.5.

blue as mediator. To this, 20 ml of previously enriched bacterial culture was added to the anodic chamber. Cathodic compartment was loaded with 2 litre of 50 mM K_2HPO_4, pH 7.5 (ref. 17). Aerated K_2HPO_4 was taken as electron acceptor at cathode[18]. The circuit was also connected to a fixed external load of 10 and 100 Ω in series[19].

Circuit assembly, measurement of potential difference and current: The two chambers were internally connected by a salt bridge and externally the circuit was connected with copper wires which were joined to the two electrodes by a multimeter. The potential difference and current output of MFC were measured using a multimeter (Mastech; model no. M830BZ). All the experiments were performed in triplicate.

MFC operations

Isolated colonies were aseptically transferred to 20 ml LB broth and incubated at 37°C/160 rpm for 12 h. The two chambers were surface-sterilized prior to operation of MFC by 70% alcohol and 1% $HgCl_2$. The MFC was operated at room temperature, i.e. 25°C under static condition. The ability of the four isolates to generate potential difference was tested in the presence of varied glucose concentrations (5–10%). The MFC was run up to 7 h and the voltage was recorded at 1 h interval in all the cases. The multimeter was connected in series for measuring the potential difference and current. The readings were noted only after the potential difference readings were constant.

Statistical analysis

All data are expressed as mean ± standard deviation (SD) of the triplicate experimental data. A two tailed Student's *t*-test was used to determine the differences in potential generation with respect to time. *P* value of <0.05 was considered significant.

Results

Biofilm assay and fluorescence microscopic analysis

Biofilm formation potential of the isolates was screened using glass tube assay. The tubes with attached cell mass visible after crystal violet staining were considered as biofilm-positive. The strains used for MFCs were found to be biofilm-positive (Figure 2 *a* and *b*). After 48 h, the maximum biofilm growth was shown by *Pseudomonas mendocina* NR802, followed by *Pseudomonas pseudoalcaligenes* NP103, *S. acidaminiphila* NCW702 and *Paenibacillus lautus* NE3B01 (Figure 2 *b*).

For fluorescence microscopy, the 48 h grown biofilm on glass slides was stained with acridine orange. A dense biofilm was observed under fluorescence microscope at liquid–air interface after 48 h of growth (Figure 3 *a–d*).

Current generation from MFC systems at different salt concentrations

The anode compartment of MFC was loaded with 20 ml of *Paenibacillus lautus* NE3B01 culture in minimal medium (1.50 litre) with glucose (5 g/l) as carbon source and 10 ml methylene blue as mediator. The other three strains were also tested one by one in the same manner. In the cathode compartment 2 litre of 50 mM K_2HPO_4, pH 7.5

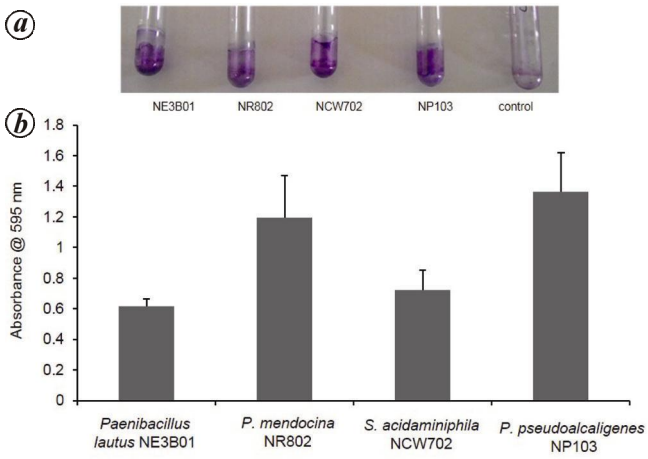

Figure 2. ***a***, Biofilm screening of the strains. Biofilm visible as attached cell mass after ethanol destaining. ***b***, Quantification of biofilm growth in terms of absorbance @ 595 nm.

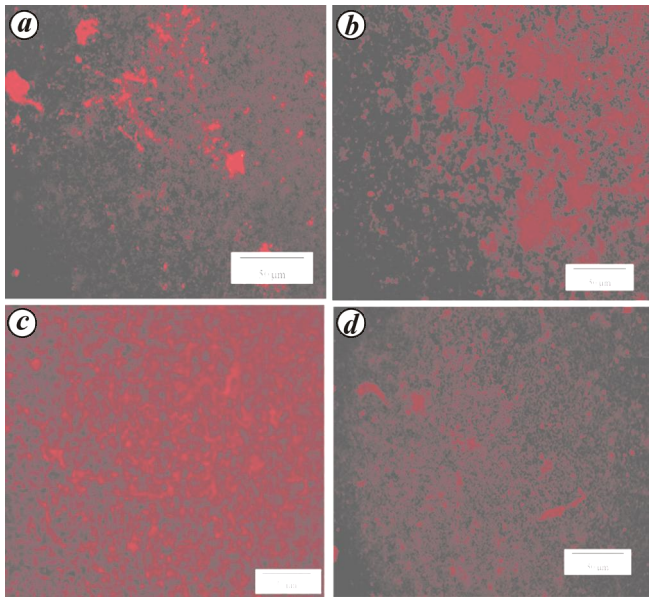

Figure 3. Fluorescence image of (***a***) *Paenibacillus lautus* NE3B01, (***b***) *Pseudomonas mendocina* NR802, (***c***) *Stenotrophomonas acidaminiphila* NCW702 and (***d***) *Pseudomonas pseudoalcaligenes* NP103.

was used. Both the compartments were connected via a salt bridge. Effect of NaCl and KCl on internal resistance of the salt bridge was observed in terms of current generation over the period of 2 h after achieving stable readings. Maximum current generation was observed when the salt bridge was constructed using 1 M KCl (267.5 ± 7.77 μA) for *Paenibacillus lautus* NE3B01 followed by *Pseudomonas mendocina* NR802, which gave a maximum current of 205 ± 5.65 μA, indicating low internal resistance (Figure 4). The current density calculated follows the same pattern and was found to be highest when the salt bridge was fabricated using 1 M KCl (8.91 ± 0.77 μA cm^{-2}) for *Paenibacillus lautus* NE3B01.

Potential difference, current generation and current density in MFC

Two compartments of MFC were connected via a salt bridge constructed with 1 M KCl, 6 g/l glucose. Potential development from the fuel cell was monitored at regular time intervals over a period of 7 h. Maximum potential was generated in the sixth hour of operation (727.5 ± 13.4 mV); thereafter, a decline in the potential was observed in case of *Paenibacillus lautus* NE3B01 (Figure 5 *a*). For *Pseudomonas mendocina* NR802, the maximum potential was generated in the seventh hour of operation (627.2 ± 5 mV). *Stenotrophomonas acidaminiphila* NCW702 and *Pseudomonas pseudoalcaligenes* NP103 also generated maximum potential of 445 ± 2.82 and 368 ± 4.2 mV in the sixth hour of operation.

Potential difference and current were measured at 0, first, third and sixth hour of growth (Table 1). Considering 0 h at reference, the *P* values for first, third, sixth and seventh hour for potential difference were 0.0023 ($P \leq 0.005$), 0.0001 ($P \leq 0.0005$), 0.0006 ($P \geq 0.0005$) and 0.047 ($P \leq 0.05$) respectively, for *Paenibacillus lautus* NE3B01; 0.0176 ($P \leq 0.05$), 0.0009 ($P \geq 0.0005$), 0.00016 ($P \leq 0.0005$) and 0.000148 ($P \leq 0.0005$) for *Pseudomonas mendocina* NR802; 0.059778 ($P \geq 0.05$), 0.024148 ($P \leq 0.05$), 0.000418 ($P \leq 0.0005$) and 0.000689 ($P \geq 0.0005$) for *Stenotrophomonas acidaminiphila* NCW702, and 0.014173 ($P \leq 0.05$), 0.000984 ($P \geq 0.0005$), 0.000585 ($P \geq 0.0005$) and 0.000896 ($P \geq 0.0005$) for *Pseudomonas pseudoalcaligenes* NP103 respectively. The finding indicates that the time for MFC operation has significantly affected potential difference generated with time as *P* value in all the case is less than 0.05, which is the significant range[17].

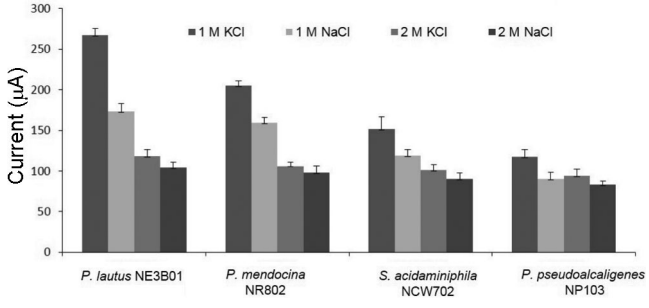

Figure 4. Effect of salt concentration on current generation.

Maximum potential difference and current output after 6 h was observed when MFC was operated with *Paenibacillus lautus* NE3B01. However, *Pseudomonas mendocina* NR802 MFC was found to be the most stable among all the strains as even after 7 h an increase in potential and current was observed. There was decline in potential generation after 6 h when MFC was operated with other strains (Figure 5 *a* and *b*). The maximum current density of 22.68 μA cm^{-2} was obtained in *Paenibacillus lautus* NE3B01. *Stenotrophomonas acidaminiphila* NCW702, *Pseudomonas pseudoalcaligenes* NP103 and *Pseudomonas mendocina* NR802 generated current densities of 13.93, 11.4 and 19.68 μA cm^{-2} respectively (Figure 5 *c*).

Effect of glucose concentration on the potential generated

The effect of different concentrations (5–10 g/l) of glucose on potential difference by the different bacterial strains was studied (Figure 6). Potential difference generated across the MFC compartment was monitored over the period of 6 h. On increasing the concentration of glucose from 5 to 6 g/l, the voltage increased by 11.74%, 19.3%, 8.07% and 1.17% in *Paenibacillus lautus* NE3B01, *Pseudomonas mendocina* NR802, *Stenotrophomonas acidaminiphila* NCW702 and *Pseudomonas pseudoalcaligenes* NP103 respectively. Further, increase in concentration from 6 to 7 g/l of glucose did not significantly increase the voltage percentage in *Stenotrophomonas acidaminiphila* NCW702. However a considerable increase in voltage of 13.4% and 5.34% in *Pseudomonas mendocina* NR802 and *Pseudomonas pseudoalcaligenes* NP103 was observed. Further increase in glucose concentration did not increase the voltage production (Table 2). Thus, 7 g/l of glucose was considered as the optimal concentration for further MFC studies.

Effect of external resistance on current output of Pseudomonas mendocina NR802

MFC of *Pseudomonas mendocina* NR802 was monitored for voltage and current output for 7 h and was observed as the most stable among all the isolates used in this study. MFC was connected in series to an external load of 10 and 100 Ω individually. The maximum current output was obtained with external load of 10 Ω. The current output at zeroth, first, third, sixth and seventh hour of operation was 25.5 ± 2.3, 104.8 ± 6.12, 149.17 ± 12.11, 253.61 ± 19 and 279.5 ± 23.14 μA respectively. In the series combination of 10 and 100 Ω external load, the current output was reduced (Figure 7).

Table 1. Potential generation (mV) by biofilm-forming marine bacteria *Paenibacillus lautus* NE3B01, *Pseudomonas mendocina* NR802, *Stenotrophomonas acidaminiphila* NCW702 and *Pseudomonas pseudoalcaligenes* NP103 MFC at different time intervals

Time (h)	*Paenibacillus lautus* NE3B01	*P* value	*Pseudomonas mendocina* NR802	*P* value	*Stenotrophomonas acidaminiphila* NCW702	*P* value	*Pseudomonas pseudoalcaligenes* NP103	*P* value
0	344.5 ± 2.1		296 ± 2		288.5 ± 3.5		177.5 ± 4.9	
1	424.5 ± 4.9	0.0023 ≤ 0.005	317 ± 2	0.017661 ≤ 0.05	301 ± 2.8	0.059778 ≥ 0.05	211 ± 2.8	0.014173 ≤ 0.05
3	694 ± 4.24	0.0001 ≤ 0.0005	414 ± 3	0.000932 ≥ 0.0005	332.5 ± 9.19	0.024148 ≤ 0.05	314.5 ± 3.5	0.000984 ≥ 0.0005
6	727.5 ± 13.4	0.0006 ≤ 0.0005	543.5 ± 2.5	0.000167 ≤ 0.0005	445 ± 2.82	0.000418 ≤ 0.0005	368 ± 4.2	0.000585 ≥ 0.0005
7	435 ± 28.9	0.047 ≤ 0.05	627.2 ± 5	0.000148 ≤ 0.0005	391 ± 1.41	0.000689 ≤ 0.05	299 ± 1.4	0.000896 ≥ 0.0005

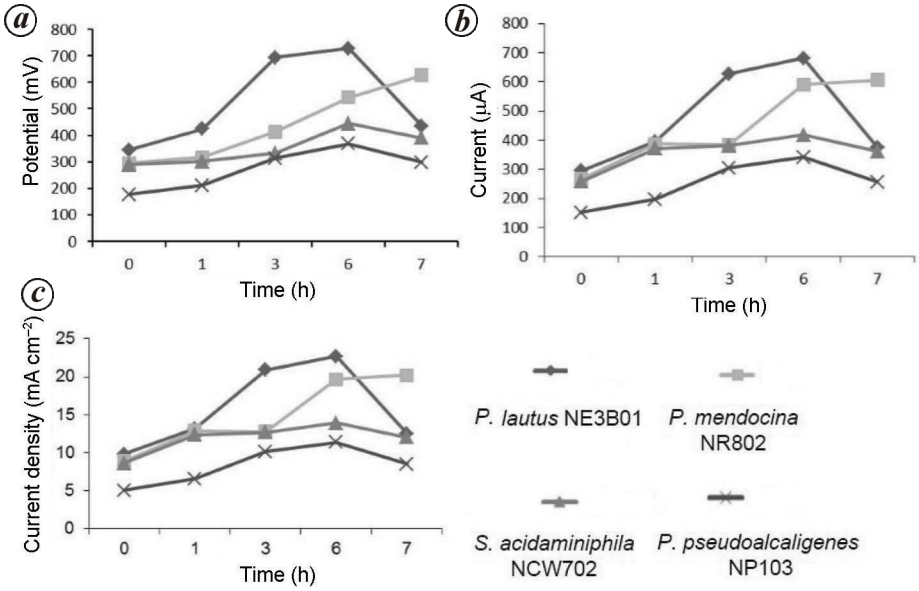

Figure 5. Potential difference and current output in MFCs with marine bacteria. ***a***, Potential difference in relation to time; ***b***, Current output in relation to time; ***c***, Current density in relation to time.

Discussion

Biofilm formation begins with initial attachment of planktonic cells to the substratum, followed by cell division, growth, maturation and detachment of cells from the community. The detached cells can again form a new biofilm after coming in contact with the substratum[20]. Several types of MFCs using different microbes have been widely studied and reviewed[1]. A basic MFC was designed and operated using marine biofilm-forming bacteria, to study their power generation and current output. Compared to planktonic forms, biofilm-forming microbes are promising target for MFC[21]. For biofilm-forming bacteria, current generated by the MFC is dependent on bacterial cell concentration on the electrode surface. The marine bacteria are capable of converting glucose (used as substrate) into electricity.

The optimum glucose concentration was 7%. *Paenibacillus lautus* NE3B01 among the four strains generated the maximum voltage of 727.5 ± 13.4 mV in 6 h. However, the MFC of *Pseudomonas mendocina* NR802 was the most stable and constant. The potential and current output by *Pseudomonas mendocina* NR802 in the seventh hour were 627.2 ± 5 mV and 605.45 ± 15 μA respectively. The output voltage and current were higher compared to previously reported mix culture MFC[22].

The electrochemical activity is believed to be due to the direct electron transfer to the electrode. Biofilm can assist in direct electron transfer to the electrode. However in the present study, MFC was operated using methylene blue as the mediator. In general, mediators are used to shuttle electrons from cell to electrode. Bacterial MFCs are more effective than yeast-mediated MFCs as *Paenibacillus lautus* was able to generate a potential of

Table 2. Effect of glucose concentration on potential generation (mV)

Glucose concentration (g/l)	*Paenibacillus lautus* NE3B01	*Pseudomonas mendocina* NR802	*Stenotrophomonas. acidaminiphila* NCW702	*Pseudomonas pseudoalcaligenes* NP103
5	794.5 ± 3.5	526 ± 4.2	224 ± 4.2	191.5 ± 2.1
6	970.5 ± 0.70	814.5 ± 6.3	345 ± 2.8	209 ± 2.8
7	1035.5 ± 3.53	1015.5 ± 7.7	368.5 ± 4.9	291 ± 5.6
8	1025 ± 2.8	999 ± 2.8	330 ± 5.6	211 ± 2.8
9	1015.5 ± 3.5	973 ± 5.6	304.5 ± 9.19	220 ± 4.2
10	995.5 ± 4.9	965 ± 5.6	297 ± 8.4	202 ± 7.0

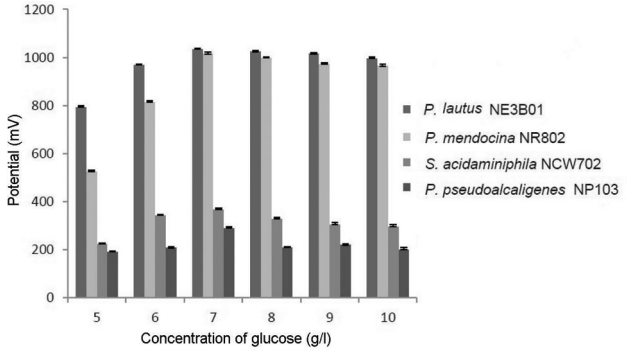

Figure 6. Effect of glucose concentration on potential generated by different bacterial strains.

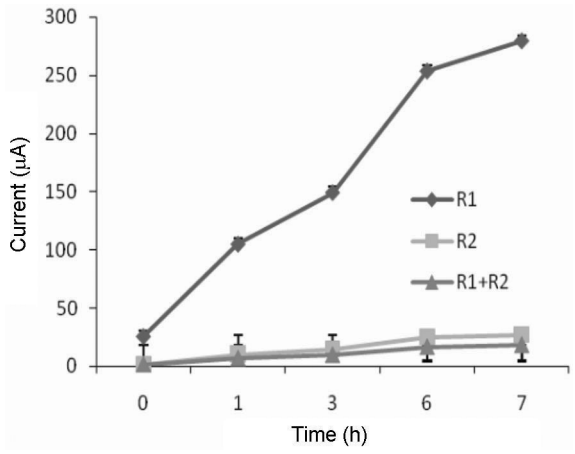

Figure 7. Effect of external load (resistance) on current output in MFC of *Pseudomonas mendocina* NR802. $R1 = 10\ \Omega$, $R2 = 100\ \Omega$, $R_1 + R_2$ in series.

727.5 ± 13.4 mV in 6 h with 7 g/l of glucose, whereas only 300 mV of potential was generated in 10 h by *Stenotrophomonas cerevisiae* MFC with 30 g/l of glucose[23]. The experimental data show the useful implication of *Paenibacillus lautus* NE3B01 in MFC technology in the future. Among the other three strains, *Pseudomonas mendocina* NR802 also showed promising results. The maximum potential difference reported by Aelterman *et al.*[24] by six MFCs connected in series was 2.02 V for high voltage generation. With simple salt bridge in 6 h, the result obtained for *Pseudomonas mendocina* NR802 MFC was 1015 mV, indicating the potential of its use in MFC.

KCl and NaCl were compared for use as strong salts in the fabrication of the salt bridge. Molar concentration of salts is critical and plays a decisive role in the translocation of protons. The results obtained were in line with a previous result[2]. Also, 1 M KCl was efficient in transporting H^+ ions. This voltage generated is comparable or better than previous results obtained using other single-chamber systems[25], but the design used here is simpler, more affordable and does not require the use of precious metals. Zhang *et al.*[26] used dairy manure as an anodic substrate and proposed improved design for biocathode MFC. They studied bacterial biofilms over anodic surface comprised principally of *Proteobacteria*. Although mixed consortium favours biofilm growth on the anode, it often harbours low number of high-power producing bacteria than that reported by Kiely *et al.*[27]. Inability to use specific substrate for growth by certain microbial species and competition with other microbes often limit power output from MFCs operated using microbial consortium. Thus, single bacteria species are valuable for MFC application[27]. Read *et al.*[28] studied the initial development and structure of biofilm, predominantly formed over the anode surface. It was concluded that biofilms over MFC electrodes allow considerable conversion capacity and opportunities for bacteria to develop biofilms on the MFC electrodes, allowing extracellular electron transfer (EET). Marine bacteria structured into a biofilm have high electroactivity[29]. Anodophilic marine bacteria develop biofilms on the anode and provide considerable conversion capacity and opportunities for mediator-less EET. Bacterial biofilms are rich in complex extracellular proteins, exopolysaccharides and uronic acids. The matrix polymer enables bacteria to form biofilms on the anode, which can potentially transport electrons. The whole biofilm acts like a living electrode and complex ecosystem where bacteria are living within a self-generated matrix that conducts the electrons[30].

The effects of operational conditions of a MFC were tested and optimized for the best performance in a double-chambered MFC. Molar concentration of salt is critical since the transfer of protons through the salt bridge is facilitated by the dissociated ions in it. The findings suggest that, with increase in molar concentration the current decreases. Better results were obtained when the salt bridge was fabricated using 1 M KCl. The molar concentration

of salt bridge has a significant effect on the transfer of protons, which is facilitated by dissociation of ions in the salt bridge. As molar conductivity of the solution increases with dilution, a decrease in current was observed at higher concentration of ions in the salt bridge[31]. The optimum glucose concentration for all strains was 7%. *Paenibacillus lautus* NE3B01 among the four strains generated the maximum voltage of 727.5 ± 13.4 mV in 6 h. However, the MFC of *Pseudomonas mendocina* NR802 was the most stable and constant. The potential and current output by *Pseudomonas mendocina* NR802 at the seventh hour were 627.2 ± 5 mV and 605.45 ± 15 μA respectively. Kim *et al.*[3] studied the MFC of *Shewanella putrefaciens* with mediator-less and proton exchange membrane. The maximum current output was about 40 μA after 5 h of operation of MFC, with an external load of 1 kΩ. In the present study, *Pseudomonas mendocina* NR802 biofilm MFC salt bridge was used. The salt bridge offers 20 times more internal resistance compared to proton exchange membrane[32]. The current output with 10, 100 and 10 + 100 Ω (series) was 253.61 ± 20, 25.36 ± 1.9 and 16.91 ± 0.76 μA respectively, after 6 h of MFC operation. The current output of *Pseudomonas mendocina* NR802 biofilm MFC was comparable with MFC of *Shewanella putrefaciens*. When the MFC was connected to an external load of 100 Ω, the current output and potential difference were higher compared to the MFC studied by Agarwal *et al.*[33], who reported 18.1 μA of current output and 187.5 mV potential difference across the two chambers connected by a salt bridge.

The current density obtained for the strains used in the study was higher in comparison to that previously reported in *Shewanella oneidensis* MR-1 (6.7 μA cm^{-2})[34] and *Geobacter*-enriched culture mediated MFC (10 μA cm^{-2})[35]. In contrast, the current density for *Paenibacillus lautus* NE3B01 was found to be 22.6 μA cm^{-2} followed by *Pseudomonas mendocina* NR802 having a maximum current density of 19.68 μA cm^{-2}. *Shewanella oneidensis* MR-1 and *Geobacter*-enriched culture mediated MFCs are micro-fabricated and their construction is complex. Nevertheless, the current generated by the relatively simple MFC constructs used in the present study is more economical than these highly intricate designs. Current output is low compared to other MFCs previously studied with proton exchange membrane. This may be attributed to the high internal resistance of the salt bridge[1]. However, the current generation is promising with the isolates used in the present study and may be useful for screening and selecting the potential anodophilic biofilm-forming marine bacteria, which can be used in MFC technology as a sustainable future energy resource.

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