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Bioelectricity production and simultaneous bio-desalination of seawater with marine bacteria

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ABSTRACT

The idea of using marine bacteria to desalinate seawater and simultaneously generate electricity from it as a sustainable resource has been investigated. To obtain electrogenic bacterial isolates with the ability to desalinate by intracellular ion transfer, extensive sampling was carried out from different regions of the Persian Gulf. The performance of the achieved strains was studied using seawater as substrate in a microbial fuel cell system. The closed-circuit voltage was measured to be 243 mV and the concentration desalination rate was 13.8 g/(L·d). In addition, the total dissolved solids value of seawater in the anode decreased by 18.7%. The ion chromatography results showed that the amount of eleven major ions decreased between 5.9% (Ca^{+2}) and 41.9% (HCO_3^-). The reduction rate of Na^+ and Cl^- ions was 3.9% and 17.4%, respectively. A practical seed sowing was performed using the desalinated water for irrigation. The seeds germinated after 10 d and leafed after 12 d. Finally, identification of bacterial strains was performed. Based on biochemical tests as well as phenotypic characteristics and 16SrRNA gene sequence, the isolates *Bacillus* sp. *IrKAM1*, *Bacillus* sp. *IrKAM2* and *Bacillus* sp. *IrKAM3* were identified as *Enterobacter ludwigii* EN–119(T), *Acinetobacter baumannii* ATCC 19606(T) and *Enterobacter sichuanensis* WCHCAI1597(T), respectively.

Keywords: Bioelectricity production; Bio-desalination; Microbial fuel cell; Seawater; Marine bacteria

1. Introduction

Global electricity consumption was 76.5 × 10^{18} J in 2017, with forecasts of 102.6 × 10^{18} J in 2030 and 151.8 × 10^{18} J by 2050 [1]. This results in a growth rate of 2.5%/y until 2030 and about 2%/y thereafter. Therefore, electric power generation from a variety of sources, especially sustainable ones, is essential.

Microbial fuel cells (MFCs) are an innovative approach of using microorganisms to generate renewable electrical energy [2]. Bacteria act as catalysts in MFCs, oxidizing organic or inorganic materials in the anodic half-cell and generate electrical current. Electrons produced by the bacteria from these substrates are transferred to the cathode chamber through a conductive channel consisting of the anode electrode, a wire, a resistor or other electrical load, and the cathode electrode. These electrons are consumed in the cathode chamber in the presence of protons using chemical catalysts [2].

In general, various microbial cells (MXCs) are novel bio-electrochemical systems equivalent to MFC, with minor variations in anode and/or cathode configuration. Microscopic microbes and chronic wastes are used. Other MXCs include microbial desalination cell (MDC) [3], microbial electrolysis cell (MEC) [4,5], microbial reverse electrodialysis cell (MRC) [6], and microbial solar cell (MSC) [7].

MDCs are three-chambers MFCs used simultaneously for organic waste treatment and saltwater desalination [8–10]. MEC produces hydrogen or methane from organic waste [5]. MRC generates electricity using the entropic energy of

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the salinity difference between seawater and river water [6]. MSC uses photosynthetic bacteria and solar energy conversion to generate electricity [7]. A variety of substrates can be provided to the bacteria to generate electrical energy through their oxidation. This method can be used to treat municipal waste streams and high strength organic wastes from livestock farms [11], landfills [12], and food processing plants [13–15].

Another vital global need is water. Due to climate change and water scarcity in the world, the supply of potable water and water resources for industrial and agricultural purposes is now a major challenge. The percentage growth of water consumption from 1900 to 2017 was about 750% and will increase to about 1,100% by 2040 [16]. About 40% of the countries in the world are located in water-scarce or dehydrated areas [17]. The population living in these areas is increasing, reaching more than two billion people today [17]. Freshwater sources include groundwater, surface water, and glacial water, which account for only 2.5% of the total water on Earth [17]. The remaining 97.5% is found in the oceans [17]. Desalination of this huge resource can be one way to provide the required water.

Conventional desalination technologies, including thermal, membrane, and hybrid methods, have made great progress to date [18]. However, these technologies have several drawbacks. The main problem with these processes, in addition to their high cost, is their requirement for thermal or electrical energy [18]. Also, the disposal of large amounts of saline wastewater and salt masses is a major challenge [19,20]. Providing an environmentally friendly system for desalination can solve these problems to an acceptable extent. There are some attempts in this direction in the literature. One example is the use of photosynthetic bacteria [21,22]. Halophytic microalgae and halophilic microorganisms have also been used for this purpose [23–25].

In the literature, MFC has been utilized to generate electricity from seawater [26]. A photosynthetic MDC has also been used to simultaneously desalinate wastewater and generate bioelectricity [27]. It is known that carbon dioxide available in the ocean reacts with seawater to produce carbonic acid, bicarbonate ions, and carbonate ions. Bicarbonate ions account for about 90% of the total organic carbon in seawater [28]. It is also evident that bacteria living in seawater have adapted to the complex biological conditions and use the energy and food sources for their metabolism [29]. Seawater and bacteria living in it could be directly used as a substrate and biocatalyst in the anode section of an MFC system, allowing simultaneous electricity generation and desalination. The electricity is generated by live bacteria oxidizing the compounds in seawater. This lowers the total dissolved solids (TDS) level of the seawater, and the produced water can be used for agricultural or industrial purposes. As a consequence, this technology can meet the demand for energy and water at the same time. Based on this hypothesis, this study investigated the generation of bioelectricity and simultaneous biodesalination in an MFC using only seawater as substrate, without additives such as acetate, etc.

Extensive seawater samples were collected from different locations in the Persian Gulf to achieve electrogenic bacteria that can be used in the MFC system and also have the ability to desalinate seawater by transferring its ions into their cells. Different culture media were used to isolate the bacteria found in the samples. The grown isolates were chosen based on a set of criteria to ensure that they met the requirements outlined above. The selected isolates were subjected to a salt tolerance test to determine which of them would be most suitable for use in the presence of seawater. Appropriate consortia for use in the MFC system were found by running an antagonistic effect test on various combinations of the isolates that passed the salt tolerance test. We then designed a simple MFC system. The functionality of this system was evaluated using the resulting consortia as the biocatalyst and the synthetic saltwater as the substrate. The superior consortium was determined based on the amount of power generated as well as the level of desalination. The system was then operated with this consortium and a seawater sample as the substrate to see how well it could generate electricity and desalinate water at the same time. Also, a practical seed planting was performed using the desalinated water. Finally, the results of morphological and biochemical identification, 16SrRNA gene sequencing, and phylogenetic tree construction for selected isolates were presented.

2. Materials and methods

2.1. Collection of samples

The seawater samples were collected from Lavar (54°41′33.715″ N, 27°6′43.879″ E), Dayyer Port (51°56′4.692″ N, 27°50′14.642″ E), Ameri port (51°6′1.266″ N, 28°30′45.733″ E), Buol Kheyr Port (51°6′2.040″ N, 28°31′51.840″ E), Jofreh (50°49′15.726″ N, 28°58′13.739″ E), and the petrochemical desalination ponds in Mahshahr (41°11′16.547″ N, 30°33′17.495″ E), all from Persian Gulf, Iran. Samples were collected and stored at 4°C until employed in the laboratory.

2.2. Culture media and isolation of the bacteria from the samples

To isolate bacteria from the water samples, they were grown in different culture media, including nutrient (Sigma-Aldrich), Luria-Bertani (LB; Sigma-Aldrich), trypticase soy broth (TSB; Sigma-Aldrich), and a modified medium for halophiles (MH). MH was prepared with different total salt concentrations (Table 1). All samples were examined microscopically. After preparation of the stocks, the grown isolates were screened based on some parameters such as negative or positive gram bacilli without spores (the presence of spores is not suitable for biofilm formation) and facultative anaerobes (for use in anaerobic conditions in the anode chamber of the MFC). The selected isolates were used for the remainder of the study.

2.3. Salt tolerance test

Bacteria were grown in their specific broth culture media. Samples were then reached to half-McFarland turbidity (colony forming unit (CFU)). The assay was performed with 96-well microliter plates and by preparing NaCl salt stock at various concentrations from 40 to 190 g/L. In this way, 240 μL of the culture media were first added to the microliter plate wells. Then, 50 μL of the different salt

Table 1
Composition of two modified MH culture media

MH 7.5% (g/L)		MH 10% (g/L)	
Part	Weight (g)	Part	Weight (g)
$MgCl_2$	5.25	$MgCl_2{\cdot}6H_2O$	7
$MgSO_4{\cdot}7H_2O$	7.20	$MgSO_4{\cdot}7H_2O$	8
$CaCl_2$	0.27	$CaCl_2{\cdot}H_2O$	0.36
KCl	1.50	KCl	2
$NaHCO_3$	0.045	$NaHCO_3$	0.06
Yeast extract	10	Yeast extract	1
NaBr	0.0175	NaBr	0.026
Glucose	1	Glucose	1
NaCl	60.75	NaCl	80
Protease peptone	5	Protease peptone	5

concentrations were added. Subsequently, 10 μL of the bacterial suspensions were inoculated. Non-pathogenic *E. coli* ATTC25922 was also used as a negative control. The test result was monitored after 24 h.

2.4. Antagonistic effect test

This test was performed according to cross-streak method [30]. Different combinations of the isolates which satisfied the salt tolerance test were examined. Each of these combinations contained the bacterial isolates with the same growth curve. For different combinations, related culture plates were prepared and inoculated with the species of the isolates through a single inoculum streak in the center of the plate. After incubation at 37°C for 24 h, the plates were seeded with the other isolates of the combination through a single streak at 90° to the first isolate. Microbial interactions were analyzed by comparing the size of the zone of inhibition, and finally the suitable consortia were determined.

2.5. MFC construction

A simple solution was chosen for fast and low-cost construction. The designed MFC system consists of two containers connected by a bridge. Each of these containers has a dimension of 10 cm × 10 cm × 10 cm. The interface is a salt bridge consisting of 5 M sodium chloride and 10% agarose. It is known that the length and cross-section of the bridge affect the performance of the system. Since a shorter length and a larger cross-section increase the efficiency of the system, the diameter of the bridge and its length were set to 3 and 2 cm (the minimum length that can be fabricated), respectively. A basket-shaped titanium with dimensions of 6 cm × 6 cm × 6 cm was used as the electrode in the anode chamber. The anode electrode contains 300 g of granular activated carbon (GAC), which serves as a bed for the formation of a bacterial biofilm. The cathode electrode was also made of aluminium in a pocket shape with dimensions of 4 cm × 12 cm. To operate the system, anaerobic conditions must be created in the anode chamber and aerobic conditions in the cathode section. For this purpose, an air pump is used in the cathode chamber for aeration. Also, copper wires and an electrical resistance of 100 ohms were used to establish a closed circuit between the anode and cathode chambers. To achieve this design with acceptable performance, two revisions were made from Revision 1 and Revision 2 to final revision.

2.6. Formation of bacterial biofilm

To prepare the bacterial biofilm, the titanium basket containing 300 g of GAC was placed in a container with broth nutrient medium and 4.5 g/L sodium chloride. Then, half Mc-Farland suspension of bacteria isolates of each selected combination was inoculated into the nutrient medium. The vessel was placed in an incubator (GALLENCAMP, Plus ll Incubator) at 37°C for 20 d.

2.7. Operation of the MFC system with synthetic seawater to determine the superior consortium

The main MFC systems of different consortia and the control system without bacterial biofilm were operated simultaneously. The anode section contains synthetic seawater with a concentration of 40 g/L NaCl as salt and 1 g/L acetate as carbon source. The cathode section contains urban water with a TDS of 0.3 g/L. An air pump was used for aeration in this section. The only difference between the main systems and the control system is that the GACs in the anode chamber of the control system don't have a bacterial biofilm, as mentioned earlier. During the running process, the closed-circuit voltage and also the reduction of TDS level in the anode of the MFCs were measured and recorded within 12 h. The consortium that had the maximum voltage level and the greatest TDS reduction was determined to be the superior consortium.

2.8. Assessment of the system with seawater

After demonstrating the system's operation and determining the superior bacterial combination, it was tested with natural seawater in the anode chamber as substrate without any additives such as acetate to evaluate the hypothesis of simultaneous electricity generation and desalination. This water was collected from the Persian Gulf and had an initial TDS value of 46.3 g/L. In this test, the closed-circuit voltage and the TDS changes of seawater were examined.

In addition, the ion chromatography method (IC) was used for a more detailed measurement of the reduction degree of different ions in seawater. The amount of eleven major ions in the anode chamber seawater was measured before and after operation of the MFC system according to Standard Methods for the Examination of Water and Wastewater #4110 (American Public Health Association, American Water Works Association, Water Environment Federation, 1999). The instrument used was 930Compact IC Flex (Metrohm Company).

To ensure the transfer of ions into the bacterial cells, the intracellular content was measured before and after the operation of the system. For this purpose, the method of atomic absorption spectroscopy (AAS) was used. The instrument used was SpectrAA 220 (Varian, Inc.). To do this, a sample of 1 g of anode chamber GAC was taken and the

bacteria contained in it were isolated. Then, the intracellular content of these bacteria was extracted according to the protocol presented by Szatmari et al. [31]. Two control samples (25 and 50 ppm) were also used to check the accuracy of the measurement.

2.9. Stacked test and seed planting

To simulate a real factory, a stacked test was defined and implemented. Three systems were installed in stacked together. Each system has its own bacterial biofilm. The desalinated output water from the first system is fed into the anode chamber of the second-one to be further desalinated. Then, it is fed into the third system for the final desalination process. In this test, the overall reduction in TDS was measured.

The function of a real factory is very similar to this test, but the number of stacks is many more there. In this factory, there must also be a biofilm production line to provide the required biocatalysts for different systems and replace them after each run. So, the final desalinated water (output of the factory) will have a TDS value suitable for agricultural and industrial applications.

A simulated desalinated water for the factory was prepared by diluting the seawater seven times to adjust the TDS value almost equal to the TDS value of the farm water, which has been measured to be about 7 g/L. Then, seed planting was performed by using this water for irrigation.

2.10. Biochemical identification

The selected isolates were subjected to five biochemical tests to identify their characteristics. These tests include oxidase, catalase, oxidation–fermentation (OF), triple sugar iron agar test (TSI) and sugar fermentation test.

2.11. Molecular identification and phylogenetic analysis of isolates

Supplemental identification of selected isolates was performed by amplification and sequencing of the 16SrRNA gene. Total DNA was extracted according to the modified Marmur method [32]. PCR reaction was carried out using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′- GGTTACCTTGTTACGACTT-3′) for each sequence [33,34]. Analysis of the sequencing results was then performed using the EZTaxon database [35,36]. A phylogenetic tree was drawn via MEGA 10.2.2 software, and molecular genetic analysis was calculated by the neighbour-joining method. The identified gene sequences were then submitted to NCBI GenBank.

3. Results and discussion

3.1. Isolation of the bacteria from the samples

Twenty-three bacterial isolates were obtained in the culture process, but only nine of them have met the criteria mentioned in section 2.2. Fig. 1 shows a microscopic view of these isolates. As can be seen in this figure, all isolates are Gram-negative bacilli without spores. As a result, they may be suitable for biofilm formation. Also, these isolates are facultative anaerobes and can be used under anaerobic conditions in the anode chamber of the MFC. These isolates were used for the remainder of the study. To determine the anaerobicity, the isolates that obscured the entire broth culture medium were selected as facultative anaerobes. Also, isolates that grew only on the surface of the medium were classified as aerobic and excluded.

3.2. Salt tolerance test

The results of this test showed that all nine isolates exhibit suitable and relatively rapid growth in high salt concentration. Isolates #1, #2, #3, #5, and #6 grew in salt concentrations up to 90 g/L. Isolates #7, #8, and #9 showed salt tolerance up to 110 g/L. Isolate #4 had the best performance, growing in a salt concentration up to 130 g/L. The negative control, the non-pathogenic *E. coli* ATTC25922, did not grow at any salt concentration.

Since water with a salinity of 40–41 g/L occupies most of the Persian Gulf region [37], all nine isolates can be used for desalination in this area.

3.3. Antagonistic effect test

The results of the cross-streak method showed that among the combinations of bacterial isolates with the same growth curve, four combinations had no inhibitory activity. The results for these combinations have been illustrated in Fig. 2. It can be clearly seen that both isolates of each combination show continuous growth at the intersections and no inhibition zones are formed. Therefore, the bacteria in each of these combinations create no antagonistic metabolites and do not inhibit the growth of the other. As a consequence, these combinations were suitable candidates for constructing the biofilm of the MFC system for electricity generation.

It is well known that pure cultures have low energy transfer efficiency compared to the mixed bacterial consortia found in marine sediments [38]. Moreover, mixed bacterial cultures provided several advantages over pure cultures-driven MFCs, including higher resistance to process disturbances, higher substrate degradation rate, lower substrate specificity, and higher power output [39]. The results of the antagonistic effect test revealed that we could benefit from adopting mixed bacterial consortia.

3.4. Formation of bacterial biofilm

To examine the progression of the biofilm generation, we observed the scanning electron microscopy images (SEM) of a sample of sterile GAC and compared them with the SEM images of the GAC sample used to generate the biofilm on the 1st, 6th, 12th, and 20th days of the formation process. These images have been shown in Fig. 3 for combination #1. Fig. 3a–d show SEM images of biofilm formation on day #1, day #6, day #12, and day #20, respectively. As illustrated in these figures, bacterial biofilm formation is clearly visible and exhibits progressive growth. Fig. 3e shows a SEM image of sterile GAC used as a negative control to confirm the biofilm formation process. It is clear that this GAC has no biofilm.

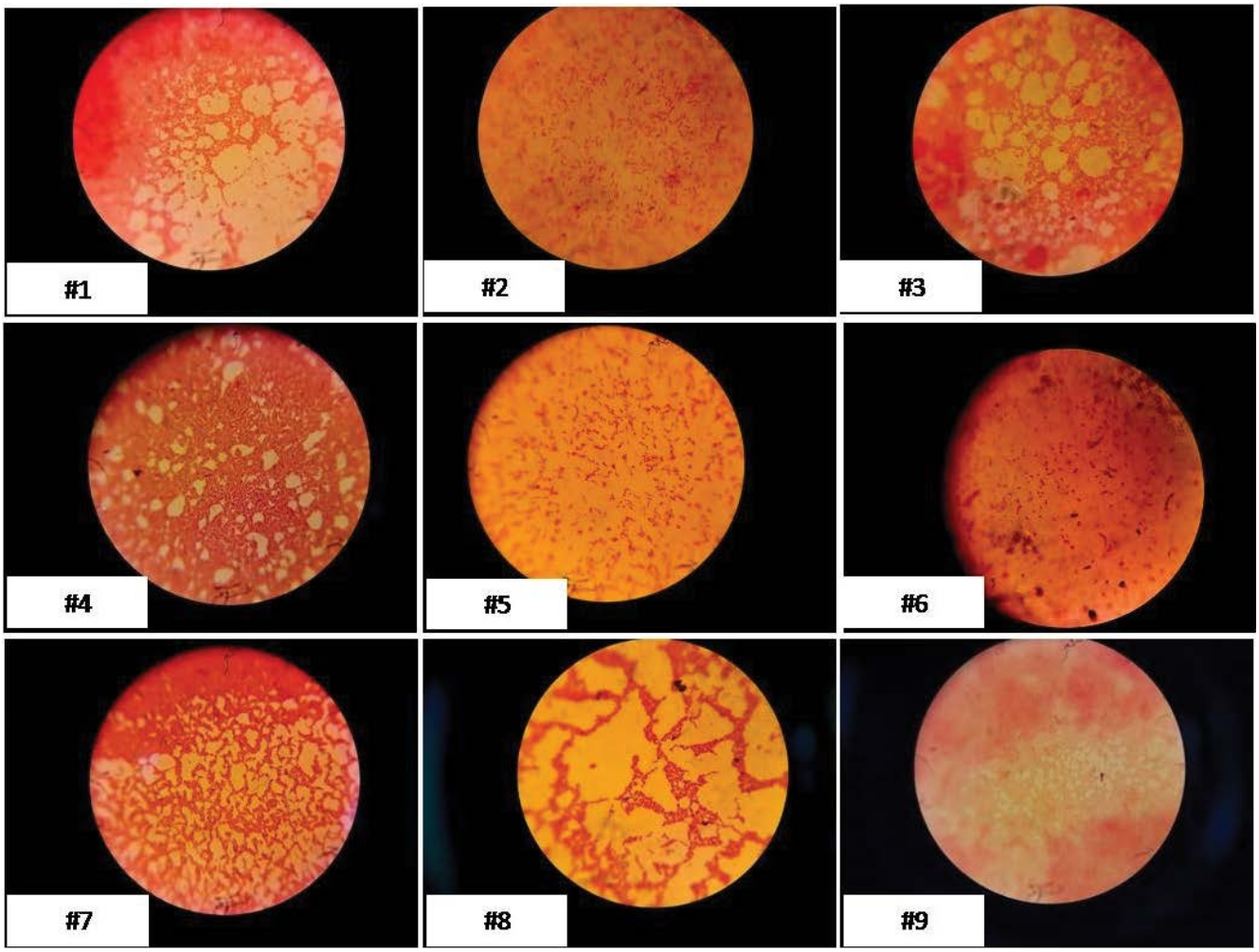

Fig. 1. Microscopic view of cultured bacterial isolates meeting the criteria in section 2.2. Gram staining shows that all nine isolates are Gram-negative bacilli.

3.5. Operation of the MFC system with synthetic seawater to determine the superior consortium

The results of the run assessments of four main systems containing bacterial consortia and the control system in 12 h have been shown in Table 2. As can be seen from this table, the maximum closed-circuit voltage of the system belongs to combination #1 which is 250 mV. Combinations No. 2, No. 3, and No. 4 produced 60, 38, and 69 mV, respectively. Combination No. 1 also had the best TDS reduction value (6.9 g/L). For the other combinations, this value was 3.8, 4.6, and 4.9 g/L, respectively. Therefore, combination No. 1 was selected as the superior consortium.

It should be noted that the voltage of the control system was below 3 mV during the 12 h. Furthermore, there was no desalination capability in this system. This concludes the successful operation of the main systems.

As illustrated in Table 2, the closed-circuit voltage of the superior consortium is 250 mV. The comparison of this voltage level with the results of other researches on the MFC has been shown in Table 3 based on the data presented by Koffi and Okabe [40]. As can be seen, the performance of the constructed system is in the moderate range. Since the goal of this study is to investigate the possibility of simultaneous electricity generation and desalination using seawater and the bacteria it contains, and not to achieve a higher performance MFC system, this system was used for the rest of the study.

This consortium also has the best TDS reduction value. The concentration desalination rate (CDR) was measured using the equation presented by Jafary et al. ([41]) to compare desalination performance. The CDR was calculated to be 13.8 g/(L·d). The assessment of the results of twelve other studies (gathered in Table 2 of [41]) shows that eleven of them have a CDR of less than 13 g/(L·d) and only one study has a CDR of 60.1 g/(L·d). Consequently, the CDR achieved in this study is a good value compared to the results obtained in the available literature.

3.6. Functional test with seawater

As shown in Fig. 4, the system was able to reduce the TDS factor by 18.7% within 4 h (Fig. 4a). In contrast, the TDS factor of the cathode hardly changed (Fig. 4b). The initial open circuit voltage of the system in the test mode with seawater was 722 mV. With the establishment of the closed

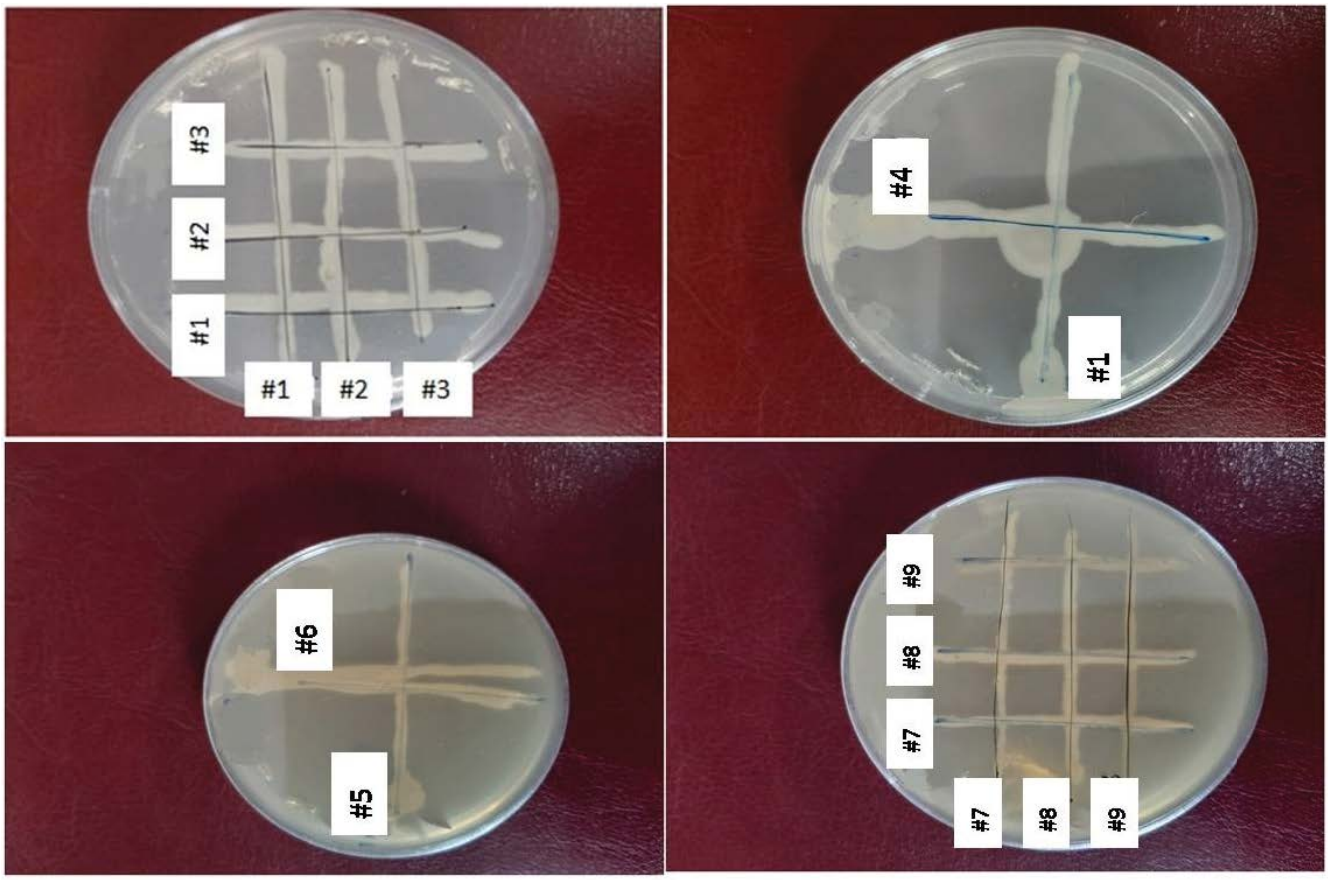

Fig. 2. The results of the cross-streak method.

circuit, the voltage started at 243 mV and reached 60 mV after 4 h (Fig. 4c). Compared to the results of the simulated seawater test reported in the previous section, these results are acceptable. This proves that the system is successful in electricity generation and simultaneous desalination when only seawater is used as substrate and no other nutrients are consumed to feed the biofilm.

The result of IC method has been presented in Table 4. As can be seen, the amount of the eleven major ions in seawater has decreased between 5.9% (Ca^{2+}) and 41.9% (HCO_3^-) after the operation of the system. The reduction rate for Na^+ and Cl^- ions were 3.9% and 17.4%, respectively.

The results from AAS for 25 and 50 ppm controls were 27 and 48.6 ppm, respectively, demonstrating the validity of the test. In addition, the results for the pre- and post-run samples were 37.4 and 44.8 ppm. This proves that the amount of ions increased after the run, which shows the transfer of ions into the bacterial cells. Therefore, it can be concluded that bacteria in the biofilm have accumulated a relatively large amount of ions in their bodies, resulting in a noticeable decrease in the TDS value of the seawater.

3.7. Stacked test and seed planting

The results of stacked test showed that the TDS value of the desalinated output water of the third system was reduced from 46.3 to 28 g/L (39.6% reduction rate). Based on this reduction rate, it can be concluded that the average reduction rate of each system was about 15.5%.

It is known the TDS value of water used for irrigation in a farm is about 7 g/L. Therefore, a reduction rate of 85% is required to reduce the TDS value of seawater from 46.3 to 7 g/L. From the stacked test result, it can be concluded that a real factory with ten stacked systems where each system has a reduction rate of 20% is achievable. This factory has an overall reduction rate of about 90%. Therefore, its desalinated water can be used for irrigation purposes.

The water used for seed planting was prepared by diluting seawater by seven times. In other words, the TDS value of the water was reduced from 46.3 to 6.6 g/L. Then, the pepper seeds were planted in three pots. The first pot was irrigated with urban water, the second with farm water, and the last with the simulated water. Fig. 5 shows that all three pots have germinated after 10 d. Also, the first and third pots have leafed after 12 d. This result confirms that the desalinated water can be used for agricultural purposes.

3.8. Biochemical identification

The results of the biochemical tests on the bacterial isolates of the superior consortium are listed in Table 5. The three isolates of this consortium are designated as *Bacillus* sp. *IrKAM1*, *Bacillus* sp. *IrKAM2*, and *Bacillus* sp. *IrKAM3*. These isolates are all oxidase and catalase positive. OF test results

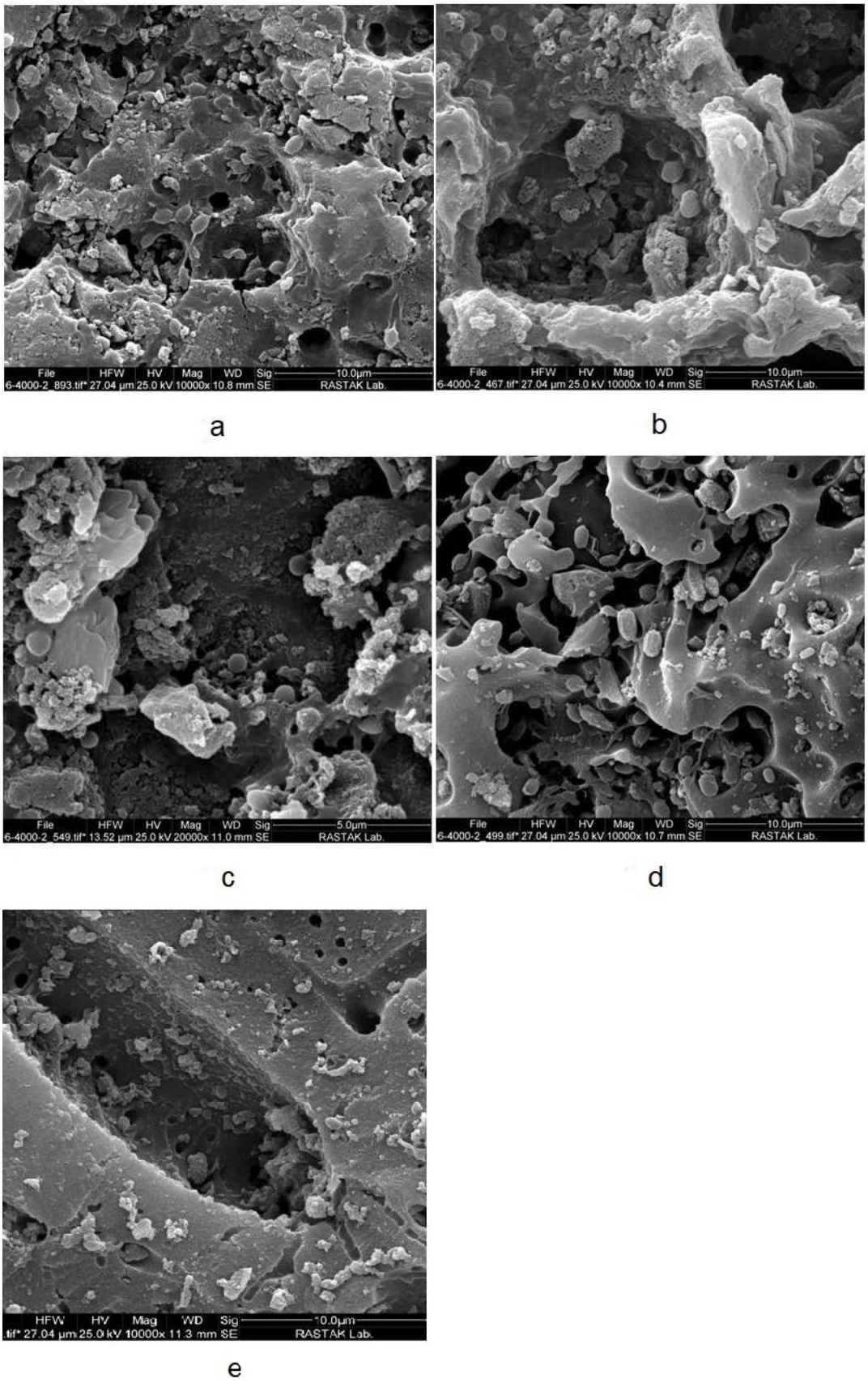

Fig. 3. SEM images of the surface of GACs containing bacterial biofilm. Images were taken on (a) 1st day, (b) 6th day, (c) 12th day, and (d) 20th day of biofilm formation. Part (e) shows SEM photo of sterile GAC.

indicate that the isolates are facultative anaerobes. According to TSI test results, the *Bacillus* sp. *IrKAM1* and *Bacillus* sp. *IrKAM3* isolates are acidic slant/acidic butt (A/A); Yellow/Yellow. In other words, they can ferment glucose, lactose and sucrose. Also, *Bacillus* sp. *IrKAM2* is alkaline slant/slant butt (K/K); Red/Red. Therefore, it cannot ferment glucose, lactose and sucrose.

Table 2
Results of the operation of the MFC system with the selected consortia. Consortium #1 has the best performance

Maximum closed-circuit voltage		Anode TDS	
Combination # (mv)		Combination # (g/L)	
33.1	1	250	1
36.2	2	60	2
35.4	3	38	3
35.1	4	69	4
40	Control system	3	Control system

3.9. Molecular identification of the superior consortium isolates

Based on the results of 16S rRNA gene sequencing, the *Bacillus* sp. *IrKAM1* strain with 1371 nucleotides was 99.6% phylogenetically identical to *Enterobacter ludwigii* with accession number EN-119(T). The *Bacillus* sp. *IrKAM2* strain with 1381 nucleotides showed 99.8% sequence similarity with *Acinetobacter baumannii* with accession number ATCC 19606(T). Finally, the *Bacillus* sp. *IrKAM3* strain with 1018 nucleotides was 99.8% phylogenetically related to *Enterobacter sichuanensis* with accession number WCHCAI1597(T). The phylogenetic tree of these strains is shown in Fig. 6.

Table 3
Comparison of the MFC performance

MFC reactor type	MFC working voltage	References
Two-chamber MFCs (1.2 mL)	0.2–0.4 V	Ref. 20 of Koffi and Okabe (2020)
Two-chamber MFCs (120 mL)	0.837 V	Ref. 10 of Koffi and Okabe (2020)
Two-chamber MFCs (50 mL)	<0.2 V	Ref. 6 of Koffi and Okabe (2020)
Air–cathode MFC (27 mL)	~0.5 V	Ref. 7 of Koffi and Okabe (2020)
Sediment microbial fuel cells (550 mL anolyte, 445 mL catholyte)	0.4 V	Ref. 11 of Koffi and Okabe (2020)
Two-chamber MFCs (150 mL)	0.310 V	Ref. 12 of Koffi and Okabe (2020)
Two-chamber MFCs (150 mL)	0.311 V	Ref. 12 of Koffi and Okabe (2020)
Single-chamber air–cathode MFC (316 mL)	0.3 V	Ref. 2 of Koffi and Okabe (2020)
Benthic MFC	0.35–0.5 V	Ref. 13 of Koffi and Okabe (2020)
Air–cathode MFC (450 mL)	0.44 V	Ref. 14 of Koffi and Okabe (2020)
Single-chamber air–cathode MFC (316 mL)	0.18 V	Ref. 22 of Koffi and Okabe (2020)
Single-chamber air–cathode MFC (300 mL)	0.2–0.5 V	Ref. 16 of Koffi and Okabe (2020)
Sediment MFC (15 L)	0.2 V	Ref. 15 of Koffi and Okabe (2020)
Single-chamber air–cathode MFC (300 mL)	0.2–0.5 V	Koffi and Okabe [40]
Two-chamber air–cathode MFC (500 mL)	0.250 V	This study

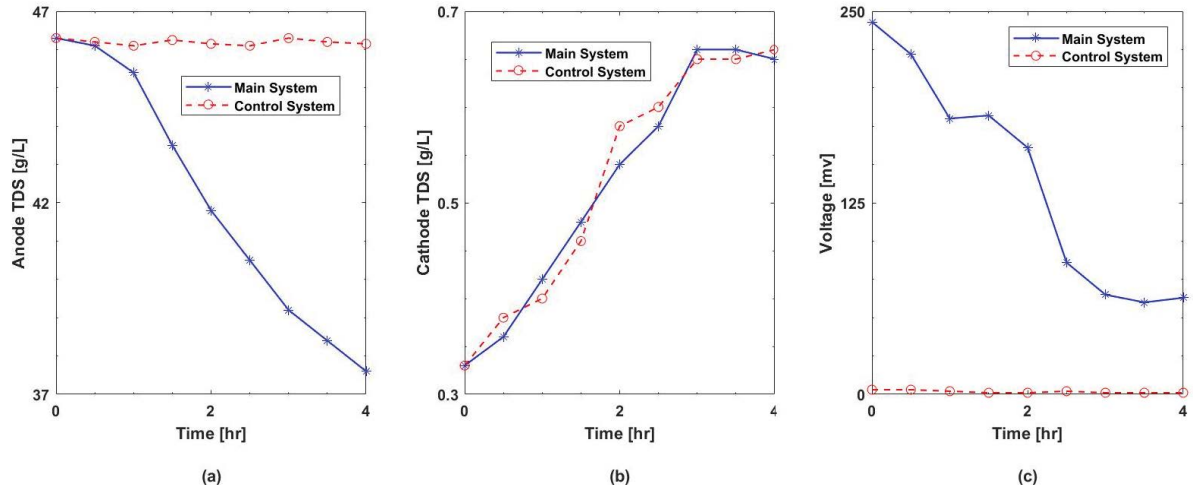

Fig. 4. Results of the functional test with seawater: (a) anode TDS, (b) cathode TDS, and (c) closed-circuit voltage.

Table 4
Results of the ion chromatography method

Ion	Concentration after test (ppm)	Concentration before test (ppm)	Reduction rate (%)
Na^{+}	11,220	10,780	3.9
Cl^{-}	20,735	17,119	17.4
SO_4^{-2}	3,123	2,523	19.2
Alkalinity	4,300	2,500	41.9
CO_3^{-2}	0.0	0.0	–
HCO_3^{-}	4,300	2,500	41.9
Ca^{+2}	681.4	641.3	5.9
Mg^{+2}	1,433	1,117	22.1
K^{+}	479.6	424.4	11.5
Sr^{+2}	3.9	2.9	25.6
Br^{-}	54	39	27.8

Table 5
Results of the biochemical tests

Biochemical test	Bacteria		
	Bacillus sp. IrKAM1	Bacillus sp. IrKAM2	Bacillus sp. IrKAM3
Oxidase	+	+	+
Catalase	+	+	+
OF	+	+	+
TSI	A/A Gas + H_2S^{-}	K/K Gas + H_2S^{-}	A/A Gas + H_2S^{-}
Glucose	+	–	+
Lactose	+	–	+
Sucrose	+	–	+

OF represents "oxidation–fermentation";
TSI represents "triple sugar iron agar".

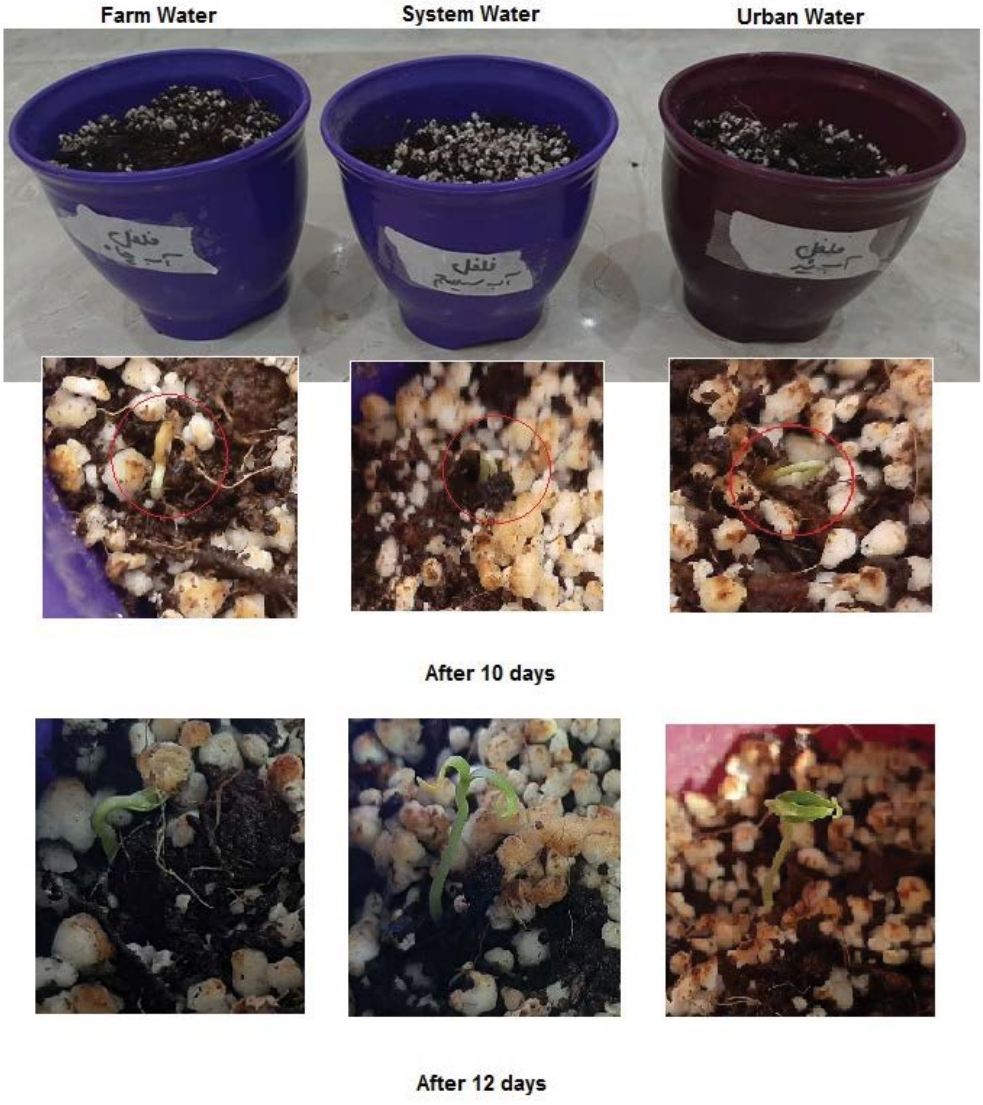

Fig. 5. Seed planting results. The pots were irrigated by urban water, farm water, and simulated water. All three pots germinated after 10 d. The pots with urban water and simulated water leafed after 12 d.

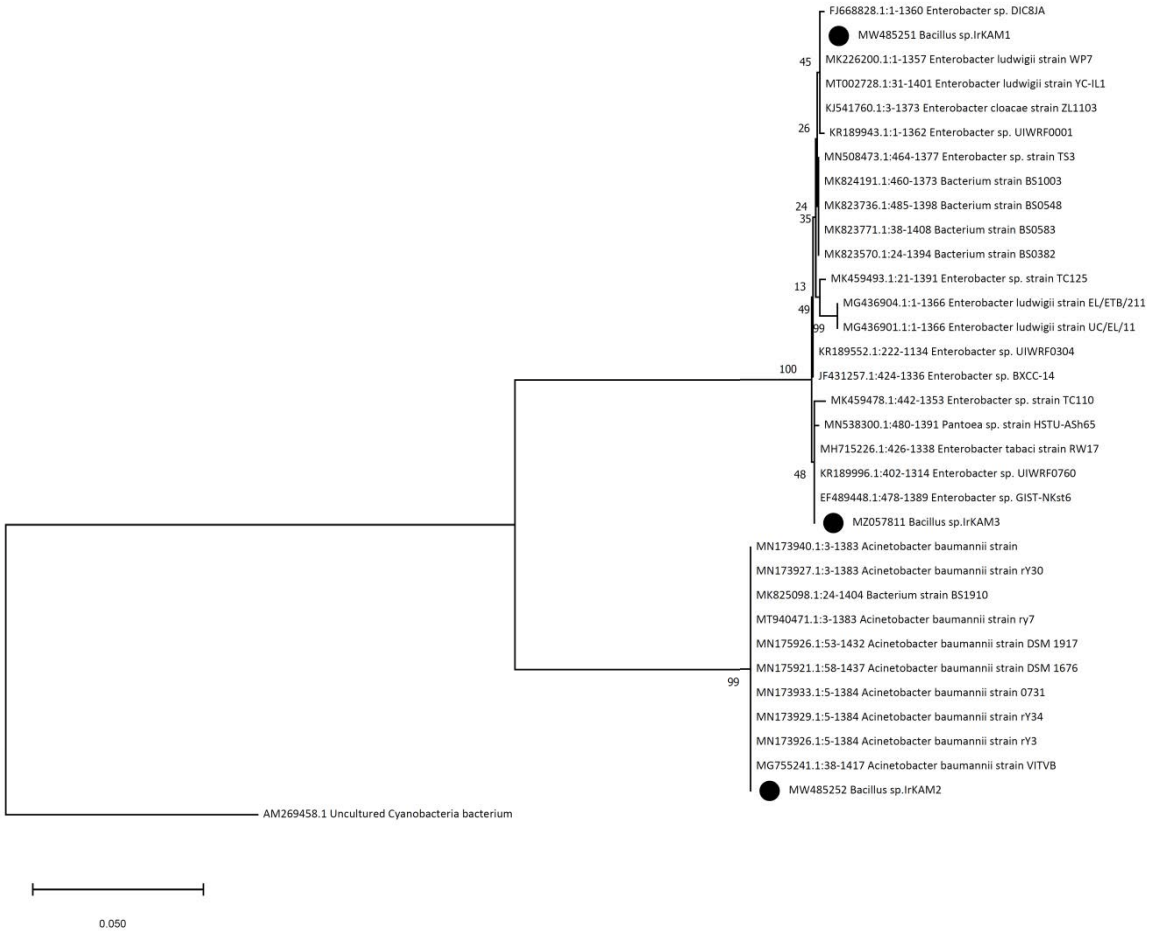

Fig. 6. Phylogenetic tree of the identified isolates.

According to NCBI data (https://www.ncbi.nlm.nih.gov/nuccore/JTLO01000001.1), *Enterobacter ludwigii* with accession number EN-119(T) and *Enterobacter sichuanensis* with accession number WCHCAI1597(T) possess Na^+ translocator protein complex. This is further evidence of the ability of the obtained bacteria to desalinate water by transferring ions into the cell.

4. Conclusion

Simultaneous electricity generation and seawater desalination in a simple MFC was achieved. Electrogenic bacterial isolates capable of desalination by intracellular ion transfer were obtained through extensive sampling from various regions of the Persian Gulf. The closed-circuit voltage reached a maximum of 243 mV and the CDR was 13.8 g/(L·d) during the system operation using only seawater as substrate in the anode chamber. The ion chromatography and AAS results confirmed the desalination performance, where the amount of eleven major ions decreased between 5.9% (Ca^{+2}) and 41.9% (HCO_3^-). The reduction rate for Na^+ and Cl^- ions were 3.9% and 17.4%, respectively. As evidenced by stacked test and practical seed planting, seawater was desalinated to less than 7 g/L, so it can be used in agriculture.

Biochemical tests as well as phenotypic characteristics and 16SrRNA gene sequence showed that the isolates *Bacillus* sp. *IrKAM1*, *Bacillus* sp. *IrKAM2*, and *Bacillus* sp. *IrKAM3* were identified as *Enterobacter ludwigii* EN-119(T), *Acinetobacter baumannii* ATCC 19606(T), and *Enterobacter sichuanensis* WCHCAI1597(T), respectively.

For further studies, it is proposed to investigate the efficacy of the system by using all microorganisms in seawater to produce the biofilm instead of employing only isolated strains. In this case, the required bed can be placed in the sea and the biofilm can be formed there as well. A scale-up MFC system [42] can also be built to determine the maximum possible desalination capacity.

CRediT authorship contribution statement

Esmat Kokabi: Investigation, Methodology, Formal analysis, Writing – original draft.

Ezat Asgarani: Supervision, Conceptualization, Validation.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration

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References

[1] International Atomic Energy Agency, 2018, Energy, Electricity and Nuclear Power Estimates for the Period up to 2050, Reference Data Series, IAEA-RDS-1/38.
[2] K. Obileke, H. Onyeaka, E.L. Meyer, N. Nwokolo, Microbial fuel cells, a renewable energy technology for bio-electricity generation: a mini-review, Electrochem. Commun., 125 (2021) 107003, doi: 10.1016/j.elecom.2021.107003.
[3] L.K.S. Gujjala, D. Dutta, P. Sharma, D. Kundu, D.-V.N. Vo, S. Kumar, A state-of-the-art review on microbial desalination cells, Chemosphere, 288 (2022) 132386, doi: 10.1016/j.chemosphere.2021.132386.
[4] B. Tartakovsky, M.F. Manuel, H. Wang, S.R. Guiot, High rate membraneless microbial electrolysis cell for continuous hydrogen production, Int. J. Hydrogen Energy, 34 (2009) 672–677.
[5] K. Sasaki, M. Morita, D. Sasaki, S. Hirano, N. Matsumoto, N. Ohmura, Y. Igarashi, Methanogenic communities on the electrodes of bioelectrochemical reactors without membranes, J. Biosci. Bioeng., 111 (2011) 47–49.
[6] R.D. Cusick, Y. Kim, B.E. Logan, Energy capture from thermolytic solutions in microbial reverse-electrodialysis cells, Science, 335 (2012) 1474–1477.
[7] J.M. Pisciotta, Y. Zou, I.V. Baskakov, Light-dependent electrogenic activity of cyanobacteria, PLoS One, 5 (2011) e10821, doi: 10.1371/journal.pone.0010821.
[8] T. Jafary, A. Al-Mamun, H. Alhimali, M.S. Baawain, S. Rahman, W.A. Tarpeh, B.R. Dhar, B.H. Kim, Novel two-chamber tubular microbial desalination cell for bioelectricity production, wastewater treatment and desalination with a focus on self-generated pH control, Desalination, 481 (2020) 114358, doi: 10.1016/j.desal.2020.114358.
[9] M. Zahid, N. Savla, S. Pandit, V.K. Thakur, S.P. Jung, P.K. Gupta, R. Parsad, E. Marsili, Microbial desalination cell: desalination through conserving energy, Desalination, 521 (2022) 115381, doi: 10.1016/j.desal.2021.115381.
[10] S. Rahman, S.A. Siddiqi, A. Al-Mamun, T. Jafary, Sustainable leachate pre-treatment using microbial desalination cell for simultaneous desalination and energy recovery, Desalination, 532 (2022) 115708, doi: 10.1016/j.desal.2022.115708.
[11] Y. Lee, N. Nirmalakhandan, Electricity production in membrane-less microbial fuel cell fed with livestock organic solid waste, Bioresour. Technol., 102 (2011) 5831–5835.
[12] J. Greenman, A. Gálvez, L. Giusti, I. Ieropoulos, Electricity from landfill leachate using microbial fuel cells: comparison with a biological aerated filter, Enzyme Microb. Technol., 44 (2009) 112–119.
[13] S.A. Patil, V.P. Surakasi, S. Koul, S. Ijmulwar, A. Vivek, Y.S. Shouche, B.P. Kapadnis, Electricity generation using chocolate industry wastewater and its treatment in activated sludge based microbial fuel cell and analysis of developed microbial community in the anode chamber, Bioresour. Technol., 100 (2009) 5132–5139.
[14] S.E. Oh, B.E. Logan, Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies, Water Res., 39 (2005) 4673–4682.
[15] A.P. Borole, C.Y. Hamilton, In: V. Shah, Ed., Emerging Environmental Technologies, Volume II, Springer, Dordrecht, 2010, pp. 97–113. Available at: https://doi.org/10.1007/978-90-481-3352-9_5
[16] M.W. Shahzad, M. Burhan, L. Ang, K.C. Ng, Energy-water-environment nexus underpinning future desalination sustainability, Desalination, 413 (2017) 52–64.
[17] Water Scarcity Clock, 2021. Available at: https://www.worldwater.io (Accessed July 2021).
[18] H.T.D. Thi, T. Pasztor, D. Fozer, F. Manenti, A.J. Toth, Comparison of desalination technologies using renewable energy sources with life cycle, PESTLE, and multi-criteria decision analyses, Water, 13 (2021) 3023, doi: 10.3390/w13213023.
[19] A.H. Galama, Ion Exchange Membranes in Seawater Applications Processes and Characteristics, Ph.D. Thesis, Wageningen University, Wageningen, 2015.
[20] S. Bucs, Biofouling in Reverse and Forward Osmosis Membrane Systems, Ph.D. Thesis, Delft University of Technology, 2017.
[21] J.M. Amezaga, A. Amtmann, L. Lawton, M.A. Madsen, K. Minas, M.R. Templeton, biodesalination: a case study for applications of photosynthetic bacteria in water treatment, Plant Physiol., 164 (2014) 1661–1676.
[22] K. Minas, E. Karunakaran, T. Bond, C. Gandy, A. Honsbein, M. Madsen, J. Amezaga, A. Amtmann, M.R. Templeton, C.A. Biggs, L. Lawton, Biodesalination: an emerging technology for targeted removal of Na^+ and Cl^- from seawater by cyanobacteria, Desal. Water Treat., 55 (2015) 2647–2668.
[23] M. Maru, E. Sahle-Demessie, F. Zewge, A review on bio-desalination using halophytic microalgae: opportunities and challenges, Aqua, (2021), doi: 10.2166/aqua.2021.068.
[24] L.C. Castillo-Carvajal, B.E. Barragan-Huerta, J.L. Snaz-Martin, Biodegradation of organic pollutants in saline wastewater by halophilic microorganisms, Environ. Sci. Pollut. Res., 21 (2014) 9578–9588.
[25] E. Sahle-Demessie, A. Aly Hassan, A.M. EI Badawy, Bio-desalination of brackish and seawater using halophytic algae, Desalination, 465 (2019) 104–113.
[26] S. Kumar, H.D. Kumar, K.G. Babu, A study on bioelectricity generation from the sea water using microbial fuel cell, Int. J. Curr. Res. Rev., 4 (2012) 65–72.
[27] D. Bejjanki, K. Muthukumar, T.K. Radhakrishnan, A. Alagarsamy, A. Pugazhendhi, S.N. Mohamed, Simultaneous bioelectricity generation and water desalination using *Oscillatoria* sp. as biocatalyst in photosynthetic microbial desalination cell, Sci. Total Environ., 754 (2021) 142215, doi: 10.1016/j.scitotenv.2020.142215.
[28] A.G. Dickson, In: U. Riebesell, V.J. Fabry, L. Hansson, J.-P. Gattuso, Eds., Guide to Best Practices for Ocean Acidification Research and Data Reporting, Publications Office of the European Union, Luxembourg, 2010, pp. 17–40.
[29] J.E. Hallsworth, Water is a preservative of microbes, Microb. Biotechnol., (2021) 1–24, doi: 10.1111/1751-7915.13980.
[30] M.T. Madigan, J.M. Martiko, J. Parker, Brock Biology of Microorganisms, Prentice-Hall International Inc., New Jersey, 1997.
[31] D. Szatmari, P. Sarkany, B. Kocsis, T. Nagy, A. Miseta, S. Barko, B. Longauer, R.C. Robinson, M. Nyitrai, Intracellular ion concentrations and cation-dependent remodelling of bacterial MreB assemblies, Sci. Rep., 10 (2020) 12002, doi: 10.1038/s41598-020-68960-w.
[32] J. Marmur, A procedure for the isolation of deoxyribonucleic acid from microorganisms, J. Mol. Biol., 3 (1961) 208–218.

[33] J.A. Frank, C.I. Reich, S. Sharma, J.S. Weisbaum, B.A. Wilson, G.J. Olsen, Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes, Appl. Environ. Microbiol., 74 (2008) 2461–2470.
[34] Y.L. Chen, C.C. Lee, Y.L. Lin, K.M. Yin, C.L. Ho, L. Tsunglin, Obtaining long 16S rDNA sequences using multiple primers and its application on dioxin-containing samples, BMC Bioinf., (2015), doi: 10.1186/1471-2105-16-S18-S13.
[35] O.S. Kim, Y.J. Cho, K. Lee, S.H. Yoon, M. Kim, H. Na, S.C. Park, Y.S. Jeon, J.H. Lee, H. Yi, S. Won, J. Chun, Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species, Int. J. Syst. Evol. Microbiol., 62 (2012) 716–721.
[36] M. Kim, J. Chun, 16S rRNA gene-based identification of bacteria and archaea using the EzTaxon server, Methods Microbiol., 41 (2014) 61–74.
[37] H.D. Ibrahim, P. Xue, E.A.B. Eltahir, Multiple salinity equilibria and resilience of Persian/Arabian gulf basin salinity to brine discharge, Front. Mar. Sci., 7 (2020), doi: 10.3389/fmars.2020.00573.
[38] K. Rabaey, G. Lissens, S.D. Siciliano, W. Verstraete, A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency, Biotechnol. Lett., 25 (2003) 1531–1535.
[39] K. Rabaey, N. Boon, S.D. Siciliano, M. Verhaege, W. Verstraete, Biofuel cells select for microbial consortia that self-mediate electron transfer, Appl. Environ. Microbiol., 70 (2004) 5373–5382.
[40] N.J. Koffi, S. Okabe, High voltage generation from wastewater by microbial fuel cells equipped with a newly designed low voltage booster multiplier (LVBM), Sci. Rep., 10 (2020) 18985, doi: 10.1038/s41598-020-75916-7.
[41] T. Jafary, A. Al-Mamun, H. Alhimali, M.S. Baawain, M.S. Rahman, S. Rahman, B.R. Dhar, M. Aghbashlo, M. Tabatabaei, Enhanced power generation and desalination rate in a novel quadruple microbial desalination cell with a single desalination chamber, Renewable Sustainable Energy Rev., 127 (2020) 109855, doi: 10.1016/j.rser.2020.109855.
[42] S. Pandit, N. Salva, S.P. Jung, In: R. Abbassi, A. Kumar Yadav, F. Khan, Integrated Microbial Fuel Cells for Wastewater Treatment, Butterworth-Heinemann, 2020, pp. 349–368. Available at: https://doi.org/10.1016/B978-0-12-817493-7.00016-3