



Sodium lactate-based enrichment method for isolation of microbial strains and evaluation of bioelectricity generation in bioelectrochemical systems

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ABSTRACT

Many environmentally relevant microorganisms remain uncultured or insufficiently characterized, limiting the discovery of strains with functional potential for bioelectrochemical applications. Although sodium acetate, glucose, and other carbon sources are commonly used for microbial enrichment, the use of sodium lactate as a defined single carbon source provides a selective strategy for enriching lactate-utilizing microorganisms with potential bioelectrochemical relevance. Therefore, this study developed a sodium lactate-based enrichment strategy for isolating microbial strains from environmental samples and evaluating their bioelectricity-generation potential in bioelectrochemical systems. Bacterial isolates were identified by 16S rRNA gene sequencing. The sediment-inoculated bioelectrochemical system achieved a peak current density of $14.77 \mu\text{A cm}^{-2}$, and 20 bacterial strains were isolated, whereas the organic fertilizer-inoculated BES produced $7.91 \mu\text{A cm}^{-2}$ and yielded seven isolates. Additionally, 5, 2, and 1 strains were isolated from soil, sediment, and wastewater, respectively. Among the tested strains, *Aeromonas eucrenophila* AS-26 and *Paracitrovorax citrulli* AS-12 exhibited the highest current densities of 2.18 and $2.10 \mu\text{A cm}^{-2}$, respectively, with corresponding anode biofilm protein contents of 227.59 and 162.41 mg. These findings demonstrate that the sodium lactate-based enrichment method provides an effective approach for recovering environmental microorganisms with potential applications in bioelectrochemical systems.

1. Introduction

Microorganisms play a key role in modern biotechnology and are extensively utilized in the production of bioenergy and biofuels (Vibha and Neelam, 2012). However, most microbial strains in natural environments have not yet been isolated, and their specific functions are poorly understood. Bioelectrochemical systems (BESs) utilize mixed microbial inoculum from sources such as wastewater, soil, sediments, and anaerobic sludge. These microbial communities work together to break down complex organic substrates (Beecroft et al., 2012; Sciarria et al., 2019; Stratford et al., 2014). Nevertheless, the complexity of these communities makes it difficult to fully understand their dynamics and the specific roles of individual species in substrate oxidation and electron transfer, thereby limiting their effective application in targeted biotechnological processes. To overcome this limitation, increasing

attention has been given to the use of defined cultures (Cao et al., 2019), which offer a more controlled alternative to complex consortia and enable more predictable performance (Aiyer, 2021).

Microbial interactions in anaerobic environments, such as sludge, sediments, and wastewater, can be synergistic (Venkataraman et al., 2011), antagonistic (Powers et al., 2015), or neutral (Read et al., 2010), which complicates their characterization. This complexity poses a major challenge in BES research, particularly in isolating functional strains and identifying suitable carbon sources for their growth and activity (Qiu et al., 2022; Yu et al., 2022). Defined substrates can help overcome this limitation by selectively promoting microbial growth while enriching desired metabolic traits, serving as both nutrients and electron donors. Conventional microbial isolation methods primarily focus on supporting microbial growth and maximizing the recovery of culturable microorganisms, rather than selectively enriching

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strains with specific functional traits. Therefore, the use of a defined carbon source such as sodium lactate provides a function-oriented enrichment strategy for selectively enriching microorganisms with desired metabolic capabilities. For example, lactate supplementation facilitated the isolation of butyrate-producing bacteria from swine feces (Liu et al., 2007), while citrate or glycerol-enriched electroactive populations in single-substrate systems (Zhang et al., 2020). Similarly, substances such as acetate, lactate, or glucose have been associated with improved microbial fuel cell performance (Sarmin et al., 2021), highlighting the importance of substrate selection in the bioelectrochemical process. In addition to substrate-based enrichment, selective media have been developed specifically for the isolation of exoelectrogenic microorganisms. Nazeer and Fernando (2022) developed an MnO_2 chromogenic growth and isolation medium that selectively supported exoelectrogenic bacteria and differentiated them through MnO_2 reduction associated color change. These findings demonstrate the potential function-oriented enrichment and selective cultivation strategies for recovering microorganisms with extracellular electron transfer (EET) capabilities for complex microbial communities.

Most studies investigating the isolation of microorganisms for bioelectricity generation have used substrates such as sodium acetate (Christgen et al., 2023; Zhou et al., 2024), glucose (Raut et al., 2025) and other carbon sources. In the present study, sodium lactate was instead used as the primary enrichment substrate to isolate lactate-utilizing microbial strains from diverse environmental samples, including soil, sediment, wastewater, and BESs. For pure culture isolation from BESs, samples derived from organic fertilizer and sediment were enriched in media containing sodium lactate or sodium acetate as the primary carbon source. Bioelectrochemical performance data were used to guide the isolation of dominant strains, which were obtained from anode biofilms and identified based on their electricity generation capacity of the inoculum sources. This integrated approach provides insights into the cultivation of functional microbial strains and enables future co-culture strategies with dominant sodium lactate utilizing microorganisms such as *Shewanella oneidensis* MR-1 and others, under the same substrate conditions to investigate microbial interaction and their effects on bioelectricity generation.

2. Materials and methods

2.1. Reagents and chemicals

All chemicals and reagents used in this study were of analytical grade (purity >98%) and were purchased from Aladdin Industrial Corporation Co., Ltd., China.

2.2. Sample collection

Ten grams of soil and sediment samples were collected from Jimei District in Xiamen City, Fujian Province, and the Institute of Urban Environment, Chinese Academy of Sciences, respectively. Wastewater samples were aseptically collected in 150 mL sterile screw-cap bottles from the Institute's facilities. Commercially available organic fertilizer was obtained from local suppliers. All samples were collected in pre-sterilized bags, transported immediately to the laboratory, and stored at 4 °C to preserve microbial viability before processing (De La Cruz-Noriega et al., 2023; Fayad et al., 2022; Jiang et al., 2016).

2.3. Primary bacterial screening from soil, sediment, and wastewater

One gram of soil (1% w/v), one gram of sediment (1% w/v), and 1 mL of wastewater (1% v/v) were separately mixed with 100 mL of sterile distilled water in three flasks and thoroughly homogenized. A 1 mL aliquot of the mixture was then transferred to a culture medium contained 50 mM phosphate buffer solution (pH 7.0) with the following components (g L^{-1}): 10.32, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$; 3.32, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$; 0.31,

NH_4Cl ; 0.13, KCl; 10 mM sodium lactate, 1 mL of trace mineral solution (containing per liter: 1.5 g NTA, 0.1 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.3 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.17 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g ZnCl_2 , 0.04 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.005 g $\text{ALK}(\text{SO}_4) 2 \cdot 12\text{H}_2\text{O}$, 0.005 g H_3BO_3 , 0.09 g Na_2MoO_4 , 0.12 g NiCl_2 , and 0.02 g $\text{NaWO}_4 \cdot 2\text{H}_2\text{O}$), and 1 mL of vitamin solution (containing per liter: 0.005 g 4 Aminobenzoic acid (Folate), 0.001 g D (+) biotin (Vitamin H), 0.01 g Nicotinic acid (Vitamin B3), 0.0025Ca-D (+) pantothenate (Vitamin B5), 0.025 g Pyridoxamine dihydrochloride (Vitamin B6), 0.005 g Thiaminum dichloride (Vitamin B1), 0.005 g Cyanocobalamin (Vitamin B12). The culture was incubated aerobically at 30 °C with a rotatory shaker at 150 rpm for 24 h to promote bacterial growth. A series of dilutions was made to reduce the number of cells in the samples (Jalal et al., 2005). Serial dilutions labeled as 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7} were prepared by transferring 1 mL of the homogenized sample into sterile test tubes containing 9 mL of normal saline (0.9% NaCl) and mixing thoroughly. An aliquot of 0.1 mL suspension from 10^{-5} , 10^{-6} , and 10^{-7} dilutions was poured on the surface of respective sterile agar plates in the petri dishes containing per liter: 10 mM sodium lactate, 10.32 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 3.32 g of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.31 g of NH_4Cl , 0.13 g of KCl, and trace minerals solution), and 15 g of agar powder, and was evenly spread using an L-shaped rod in triplicate. Plates were incubated aerobically at 30 °C for 24 h in an inverted position (Mukherjee and Doyle, 2025; Sankari et al., 2020).

2.4. Screening of microbial isolates from anode biofilm in BES

2.4.1. Preparation of microbial inoculum and culture media

The inoculum sources were organic fertilizer (50 g), sediment (50 g), and wastewater (50 mL), which were separately homogenized in 50 mM phosphate buffer g L^{-1} (10.32 $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$; 3.32 $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) and then sieved through laboratory gauze to use as inoculum. Two culture media were prepared with different carbon sources: 10 mM sodium lactate or 10 mM sodium acetate, 50 mM phosphate buffer solution ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 10.32 g L^{-1} ; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 3.32 g L^{-1} ; NH_4Cl , 0.31 g L^{-1} ; KCl, 0.13 g L^{-1} ; trace minerals and vitamins). The medium was purged with nitrogen gas for 30 min to remove the dissolved oxygen (Wu et al., 2020).

2.4.2. Operation of BES for isolation

A single-chamber BES (Fig. S1) with a total volume of 120 mL and an effective working volume of 100 mL was operated using a three-electrode system configuration. Carbon felt 2 cm × 2 cm, 2 cm × 3 cm, and Ag/AgCl served as working, counter, and reference electrodes, respectively (Xiao et al., 2021). A 20% (v/v) inoculum volume was transferred into the BES and operated for 10 days (240 h) using an 8-channel CHI1000C potentiostat (CHI Inc., Shanghai, China) and continuously monitored via a computer. Based on the bioelectricity production performance of the inoculum, several strains were isolated.

2.4.3. Preliminary screening of biofilm isolates

Biofilms with a size of 1 cm × 1 cm were dispersed in 50 mL of ultrapure water (You et al., 2018). Serial dilutions were made with sterilized water labeled as 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} were prepared (Jiang et al., 2018). An aliquot of 0.1 mL from the 10^{-5} , 10^{-6} , and 10^{-7} dilutions was spread onto sterile agar plates containing per liter: 10 mM sodium lactate, 10.32 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 3.32 g of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.31 g of NH_4Cl , 0.13 g of KCl, 20 g of agar powder, and trace metal solution and were streaked using an L-shaped rod and incubated aerobically at 30 °C for 24 h in an inverted position (Neetha et al., 2021).

2.5. Identification of bacterial isolates

After incubation, colonies appearing on the plates were selected for secondary screening of pure cultures. Pure cultures were obtained through repeated rounds of streaking for testing (Mishra et al., 2019;

Mukherjee and Doyle, 2025; Mulamattathil et al., 2014). The single morphological bacterial colony was transferred separately to another 0.8 mL of culture medium containing 50 mM phosphate buffer (pH 7.0) with the following components (g L⁻¹): 10.32, Na₂HPO₄·12H₂O; 3.32, NaH₂PO₄·2H₂O; 0.31, NH₄Cl; 0.13, KCl; 10 mM sodium lactate, 1 mL of trace mineral solution, and 1 mL of vitamin solution by means of inoculation loop. The culture was incubated aerobically at 30 °C in a rotary shaker at 150 rpm, and microbial growth was observed within ~48 h (Silva et al., 2020; Zhang et al., 2022).

2.5.1. PCR amplification and sequencing of the 16S rRNA gene

The 16S rRNA genes were amplified by polymerase chain reaction (PCR) using an Eppendorf Mastercycler Gradient. Universal primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-TAC GGC TAC CTT GTT ACG ACT T-3') were used as forward and reverse primers, respectively (Hamzah et al., 2020; Zhou et al., 2024). Each 50 µL PCR mixture contained 1 µL of DNA, 0.2 µL of each primer solution, 25 µL of Taq polymerase, and 23.6 µL of Millipore-filtered water. The mixture was centrifuged for 5 min before amplification. PCR was performed with an initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 20 s, annealing at 55 °C for 20 s, and extension at 72 °C for 30 s. The reaction was completed with a final extension at 72 °C for 5 min. The 16S rRNA gene of each isolate was purified and sequenced by the Biosune Biotechnology Co., Ltd. (Shanghai, China).

2.5.2. Preservation of cultures

The purified strains were initially activated in Luria-Bertani (LB) medium and incubated aerobically at 30 °C in a rotary shaker at 150 rpm. One milliliter of each bacterial culture was aseptically mixed with an equal volume of sterile 50% glycerol in cryogenic vials, and the suspensions were cryopreserved at -80 °C for experimental use (Hu et al., 2022; Li et al., 2025).

2.5.3. Phylogenetic analysis

Phylogenetic trees were constructed in MEGA 11 using the Neighbor-Joining method to infer evolutionary history (Saitou and Nei, 1987). Homologous DNA sequences were retrieved using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) 16S ribosomal RNA (Bacteria and Archaea) database. Pairwise sequence alignment was performed using ClustalW. The Maximum Composite Likelihood method (Tamura et al., 2004) was used to compute evolutionary distance in the units of number of base substitutions per site. The optimal tree was constructed using bootstrap support (1000 replicates) (Felsenstein, 1985). The analysis included 16 nucleotide sequences, comprising the target strain and 15 closely related strains. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Finally, the 16S rRNA gene sequences of the isolates were submitted to the NCBI GenBank database, where they were assigned unique accession numbers for future reference and public accessibility.

3. Evaluation of bioelectricity generation by monocultures in BES

3.1. Strains and cultivation conditions

Strains *Staphylococcus saprophyticus* AS-1 (*S. saprophyticus* AS-1), *Pantoea endophytica* AS-2 (*P. endophytica* AS-2), *Scandinavium goeteborgense* AS-3 (*S. goeteborgense* AS-3), *Staphylococcus succinus* AS-4 (*S. succinus* AS-4), *Staphylococcus succinus* AS-5 (*S. succinus* AS-5), *Staphylococcus epidermidis* AS-6 (*S. epidermidis* AS-6), *Staphylococcus edaphicus* AS-7 (*S. edaphicus* AS-7), *Enterobacter quasiroggenkampii* AS-8 (*E. quasiroggenkampii* AS-8), *Pseudomonas guariconensis* AS-9 (*P. guariconensis* AS-9), *Paenacidovorax caeni* AS-10 (*P. caeni* AS-10), *Simplicispira metamorpha* AS-11 (*S. metamorpha* AS-11), *Paracidovorax citrulli* AS-12 (*P. citrulli* AS-12), *Ralstonia syzygii* AS-13 (*R. syzygii* AS-13),

Pseudomonas guezenei AS-14 (*P. guezenei* AS-14), *Comamonas composti* AS-15 (*C. composti* AS-15), *Comamonas composti* AS-16 (*C. composti* AS-16), *Cupriavidus lacunae* AS-17 (*C. lacunae* AS-17), *Microbacterium oleivorans* AS-18 (*M. oleivorans* AS-18), *Comamonas terrigena* AS-19 (*C. terrigena* AS-19), *Enterobacter soli* AS-20 (*E. soli* AS-20), *Citrobacter pasteurii* AS-21 (*C. pasteurii* AS-21), *Delftia rhizosphaerae* AS-22 (*D. rhizosphaerae* AS-22), *Delftia acidovorans* AS-23 (*D. acidovorans* AS-23), *Delftia acidovorans* AS-24 (*D. acidovorans* AS-24), *Metapseudomonas otitidis* AS-25 (*M. otitidis* AS-25), *Aeromonas eucrenophila* AS-26 (*A. eucrenophila* AS-26), *Pseudomonas guezenei* AS-27 (*P. guezenei* AS-27), *Comamonas terrigena* AS-28 (*C. terrigena* AS-28), *Priestia megaterium* AS-29 (*P. megaterium* AS-29), *Priestia aryabhatai* AS-30 (*P. aryabhatai* AS-30), *Lysinibacillus cavernae* AS-31 (*L. cavernae* AS-31), *Microbacterium jejuense* AS-32 (*M. jejuense* AS-32), *Delftia rhizosphaerae* AS-33 (*D. rhizosphaerae* AS-33), *Comamonas fluminis* AS-34 (*C. fluminis* AS-34), and *Stenotrophomonas beteli* AS-35 (*S. beteli* AS-35) are the isolated strains used for this experiment.

All isolated strains employed in this study were maintained as refrigerated cultures. All strains were resuspended before use, and initially cultured aerobically in LB medium composed of 10 g L⁻¹ tryptone, 5 g L⁻¹ yeast extract, and 10 g L⁻¹ NaCl adjusted to 7.0 pH with NaOH or HCl before sterilization. Cultures were incubated at 30 °C in a rotary shaker at 150 rpm until the late stationary phase. Cells were harvested by centrifugation at 5000 xg for 5 min at 4 °C and washed three times with sterile 50 mM phosphate-buffered saline (PBS). The supernatant was discarded, and the cells were resuspended in the medium required for the subsequent experiments. The 2 mL cell suspensions were then transferred into an anaerobic medium containing (per liter): 10.32 g Na₂HPO₄·12H₂O, 3.32 g NaH₂PO₄·2H₂O, 0.31 g NH₄Cl, 0.13 g KCl, 20 mM sodium lactate serving as the primary carbon source and electron donor, 10 mL trace mineral solution, after autoclaving, filter-sterilized casamino acids (0.002%), 0.1 g CaCl₂·2H₂O, 0.1 g MgSO₄·7H₂O, and 1 mL vitamin solution were added. The vitamin solution was prepared according to previously described methods (Cao et al., 2023).

3.2. BES configuration and operation conditions

A three-electrode configuration was employed as a BES to evaluate bioelectricity generation by a single strain culture. All experiments were carried out under anaerobic conditions. Each three-electrode system consisted of a carbon felt working electrode (2 cm × 2 cm), carbon felt counter electrode (2 cm × 3 cm), and Ag/AgCl as the reference electrode (Saturated KCl from Shanghai Chenhua Company, China). The carbon felt electrodes, obtained from Gansu Haoshi Carbon Fibre Co Ltd., China were rinsed with acetone, cleaned with absolute ethanol, and stored in deionized water before use. Each electrochemical cell had a total volume of 120 mL and contained approximately 100 mL of sterilized culture medium as the working electrolyte. Each BES reactor was operated under continuous magnetic stirring at 50 rpm.

For each operation cycle, thirty-six groups of three-electrode system were operated, including one abiotic control without inoculation and the remaining system inoculating with strains *S. saprophyticus* AS-1, *P. endophytica* AS-2, *S. goeteborgense* AS-3, *S. succinus* AS-4, *S. succinus* AS-5, *S. epidermidis* AS-6, *S. edaphicus* AS-7, *E. quasiroggenkampii* AS-8, *P. guariconensis* AS-9, *P. caeni* AS-10, *S. metamorpha* AS-11, *P. citrulli* AS-12, *R. syzygii* AS-13, *P. guezenei* AS-14, *C. composti* AS-15, *C. composti* AS-16, *C. lacunae* AS-17, *M. oleivorans* AS-18, *C. terrigena* AS-19, *E. soli* AS-20, *C. pasteurii* AS-21, *D. rhizosphaerae* AS-22, *D. acidovorans* AS-23, *D. acidovorans* AS-24, *M. otitidis* AS-25, *A. eucrenophila* AS-26, *P. guezenei* AS-27, *C. terrigena* AS-28, *P. megaterium* AS-29, *P. aryabhatai* AS-30, *L. cavernae* AS-31, *M. jejuense* AS-32, *D. rhizosphaerae* AS-33, *C. fluminis* AS-34, and *S. beteli* AS-35. The cultivation temperature was maintained at 30 °C during the experiment, and the potential of the working electrode was set at +0.2 V (vs. Ag/AgCl) using an 8-channel CHI1000C potentiostat

Table 1

Identification of bacterial isolates from soil, sediment, and wastewater based on BLAST analysis of 16S rRNA gene sequences and phylogenetic analysis.

Source of isolation	Identified bacterial strain	Accession number	Closest reference strain	Accession number	Percentage identity
Soil	<i>S. saprophyticus</i> AS-1	PX122098	<i>Staphylococcus saprophyticus</i> GTC 843 ^T	NR_041324.1	99.52
	<i>S. succinus</i> AS-4	PX122237	<i>Staphylococcus succinus</i> subsp. <i>succinus</i> AMG-D ^T	NR_028667.1	98.82
	<i>S. succinus</i> AS-5	PX122239	<i>Staphylococcus succinus</i> subsp. <i>succinus</i> AMG-D1 ^T	NR_028667.1	98.56
	<i>S. epidermidis</i> AS-6	PX122258	<i>Staphylococcus epidermidis</i> Fusel ^T	NR_036904.1	98.27
	<i>S. edaphicus</i> AS-7	PX122260	<i>Staphylococcus edaphicus</i> CCM 8730 ^T	NR_156818.1	99.93
Sediment	<i>P. endophytica</i> AS-2	PX122226	<i>Pantoea endophytica</i> 596 ^T	NR_178843.1	98.59
	<i>S. goeteborgense</i> AS-3	PX122228	<i>Scandinavium goeteborgense</i> CCUG 66741 ^T	NR_180446.1	98.80
Wastewater	<i>E. quasiroggenkampii</i> AS-8	PX122261	<i>Enterobacter quasiroggenkampii</i> WCHECL1060 ^T	NR_179166.1	99.65

(CHI Inc., Shanghai, China), monitored by a computer.

4. Measurement of anode-attached biomass and biofilm visualization

4.1. Measurement of anode-attached biomass by the BCA assay

Anode-attached biomass was determined based on protein content using the bicinchoninic acid (BCA) assay. At the peak bioelectricity generation time, the anodes were collected from the BES reactors. The collected working electrodes were transferred into 50 mL centrifuge tube containing 5 mL phosphate buffer saline (PBS; 8 g L⁻¹ of NaCl, 0.2 g L⁻¹ of KCl, 1.42 g L⁻¹ of Na₂HPO₄, and 0.24 g L⁻¹ of KH₂PO₄, pH ≈ 7.4) and vortexed for 2–3 min to detach and resuspend the biofilm cells from the working electrode surface. The biofilm suspension (1 mL) was centrifuged at 13,000 ×g for 5 min, and the collected cell pellets were stored at –20 °C for 3 h. After a few minutes, 150 µL of 0.1 M NaOH was added to the pellets, followed by incubation in a water bath at 90 °C for 15 min to extract proteins. The samples were then cooled to room temperature for 15 min and centrifuged at 8000 ×g for 20 min. The protein concentration in the supernatant was determined using a BCA Protein Assay Kit (BOSTER, Wuhan, China) according to the manufacturer's instructions. Anode-attached biomass was estimated based on protein content calculated from a standard curve of protein concentration versus absorbance at 562 nm, measured using a microplate reader (SpectraMax M5, Molecular Devices), following a modified previously reported method (Dantanarayana et al., 2025; Tang et al., 2024).

4.2. Anode-attached biofilm visualization by scanning electron microscopy

The anode carbon felts were collected from the BES reactors, fixed with 2.5% glutaraldehyde, and stored at 4 °C for 4 h. The samples were subsequently dehydrated using a series of ethanol solutions (30%, 50%, 70%, 80%, 90%, and 100%; the final concentration was repeated twice) for 10 min each, followed by vacuum drying overnight. The dried carbon felts were cut into suitable sizes, mounted on SEM metal stubs using conductive tape, and sputter-coated with platinum prior to SEM

observation. The anode biofilm morphology was observed using scanning electron microscopy (SEM; S-4800, Hitachi) at an accelerating voltage of 3 kV (Han et al., 2024; Rosenbaum et al., 2011; Sarmin et al., 2021; Tang et al., 2024).

5. Results and discussion

5.1. Isolation of microbial strains from soil, sediment, and wastewater

Through repeated streaking on sodium lactate-enriched agar medium, eight bacterial strains were successfully isolated from diverse environmental sources. BLAST analysis of 16S rRNA gene sequences identified a diverse set of bacterial strains originating from soil, sediment, and wastewater samples (Table 1). Soil-derived isolates were predominantly affiliated with the genus *Staphylococcus*, including strains *S. saprophyticus* AS-1, *S. succinus* (AS-4 and AS-5), *S. epidermidis* AS-6, and *S. edaphicus* AS-7, showing high sequence similarity (98.27–99.93%). Two isolates obtained from the sediment were identified as *P. endophytica* AS-2 and *S. goeteborgense* AS-3, each showing more than 98% sequence identity to known reference strains. The wastewater-derived isolates, such as *E. quasiroggenkampii* AS-8, showed a high similarity with its closest reference strain. The phylogenetic relationships of the isolated strains are further illustrated in the supplementary information (Fig. S2).

5.2. Bioelectricity generation of organic fertilizer and sediment derived inocula, and isolation of microbial strains from BESs

As shown in Fig. 1a and b, BESs inoculated with mixed microflora demonstrated current generation associated with both anode-attached biofilms and planktonic cells. Among the tested inocula and carbon sources, the sediment inoculum cultured with sodium lactate (SL) achieved the highest electrochemical performance, reaching a peak current density of 14.77 µA cm⁻² at 192 h and an average current density of 4.14 µA cm⁻². In comparison, the organic fertilizer inoculum cultured with sodium lactate (OFL) reached a peak current density of 7.91 µA cm⁻² at 120 h, whereas the organic fertilizer inoculum cultured with sodium acetate (OFA) exhibited a lower peak current density of 3.72 µA

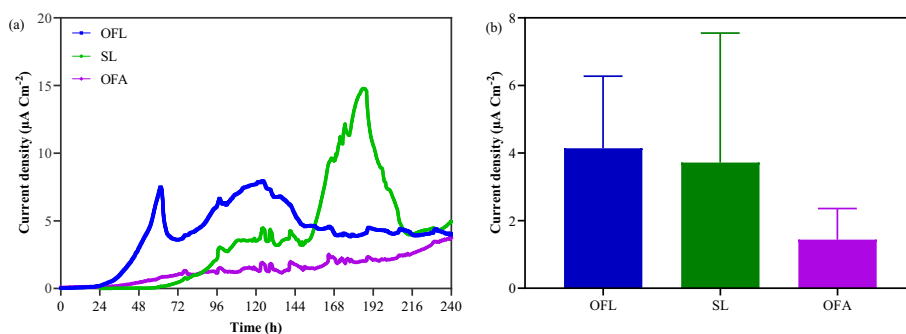


Fig. 1. Bioelectricity generation in BESs inoculated with organic fertilizer or sediment-derived inocula using lactate or acetate as carbon sources. (a, b) Current density profiles and average current density.

Table 2

Identification of bacterial strains from sodium lactate-enriched in BES and phylogenetic analysis.

Source of isolation	Identified bacterial strain	Accession number	Closest reference strain	Closest strain accession number	Percentage identity
Sediment inoculum	<i>P. guariconensis</i> AS-9	PX122328	<i>Pseudomonas guariconensis</i> PCAVU11 ^T	NR_135703.1	97.90
	<i>P. caeni</i> AS-10	PX122329	<i>Paenacidovorax caeni</i> R-24608 ^T	NR_042427.1	97.35
	<i>S. metamorpha</i> AS-11	PX122330	<i>Simplicispira metamorpha</i> DSM 1837 ^T	NR_044941.1	97.00
	<i>P. citrulli</i> AS-12	PX122331	<i>Paracidovorax citrulli</i> ATCC 29625 ^T	NR_114464.1	98.46
	<i>R. syzygii</i> AS-13	PX122332	<i>Ralstonia syzygii</i> R001 ^T	NR_025975.1	97.62
	<i>P. guezennnei</i> AS-14	PX125560	<i>Pseudomonas guezennnei</i> RA26 ^T	NR_114957.1	99.47
	<i>C. composti</i> AS-15	PX122473	<i>Comamonas composti</i> YY287 ^T	NR_044039.1	97.10
	<i>C. composti</i> AS-16	PX122474	<i>Comamonas composti</i> YY287 ^T	NR_044039.1	97.04
	<i>C. lacunae</i> AS-17	PX122672	<i>Cupriavidus lacunae</i> S23 ^T	NR_164934.1	98.47
	<i>M. oleivorans</i> AS-18	PX122671	<i>Microbacterium oleivorans</i> BAS89 ^T	NR_042262.1	97.67
	<i>C. terrigena</i> AS-19	PX122677	<i>Comamonas terrigena</i> IMI 359870 ^T	NR_028719.1	97.39
	<i>E. soli</i> AS-20	PX122676	<i>Enterobacter soli</i> ATCC BAA-2102 ^T	NR_117547.1	99.37
	<i>C. pasteurii</i> AS-21	PX122682	<i>Citrobacter pasteurii</i> CIP 55.13 ^T	NR_178769.1	99.45
	<i>D. rhizosphaerae</i> AS-22	PX122684	<i>Delftia rhizosphaerae</i> RA6 ^T	NR_157013.1	98.72
	<i>D. acidovorans</i> AS-23	PX123006	<i>Delftia acidovorans</i> ACM 489 ^T	NR_114466.1	99.06
	<i>D. acidovorans</i> AS-24	PX123010	<i>Delftia acidovorans</i> NBRC 14950 ^T	NR_113708.1	99.86
	<i>M. otitidis</i> AS-25	PX123058	<i>Metapseudomonas otitidis</i> MCC10330 ^T	NR_043289.1	99.93
	<i>A. eucrenophila</i> AS-26	PX123065	<i>Aeromonas eucrenophila</i> NCIMB 74 ^T	NR_118946.1	99.03
	<i>P. guezennnei</i> AS-27	PX123144	<i>Pseudomonas guezennnei</i> RA26 ^T	NR_114957.1	99.78
	<i>C. terrigena</i> AS-28	PX123145	<i>Comamonas terrigena</i> IMI 359870 ^T	NR_028719.1	97.21
Organic fertilizer inoculum	<i>P. megaterium</i> AS-29	PX123150	<i>Priestia megaterium</i> IAM 13418 ^T	NR_043401.1	99.17
	<i>P. aryabhattai</i> AS-30	PX123151	<i>Priestia aryabhattai</i> B8W22 ^T	NR_118442.1	100
	<i>L. cavernae</i> AS-31	PX123158	<i>Lysinibacillus cavernae</i> SYSU K30005 ^T	NR_175531.1	98.43
	<i>M. jejuense</i> AS-32	PX123168	<i>Microbacterium jejuense</i> THG-C31 ^T	NR_134085.1	98.23
	<i>D. rhizosphaerae</i> AS-33	PX123167	<i>Delftia rhizosphaerae</i> RA6 ^T	NR_157013.1	98.31
	<i>C. fluminis</i> AS-34	PX123173	<i>Comamonas fluminis</i> CJ34 ^T	NR_181645.1	97.88
	<i>S. beteli</i> AS-35	PX123227	<i>Stenotrophomonas beteli</i> ATCC 19861 ^T	NR_040804.1	99.44

cm⁻² at 240 h, with an average current density of 1.44 $\mu\text{A cm}^{-2}$. These results indicate that both inoculum source and carbon substrate may influence current generation in the BESs. Previous studies have demonstrated the suitability of lactate as a substrate for the enrichment or growth of microorganisms with relevant respiratory and electron-transfer capabilities. Huang et al. (2010), for example, enriched a lactate-fed marine sediment BES and subsequently isolated *Shewanella marisflavi* EP1. Kumar et al. (2016) also reported lactate utilization by *Tolomonas osonensis*, while (Wang et al., 2022) described lactate-fed bromate-reducing microbial consortia.

In addition to the possible effect of the carbon substrate, the higher current generation observed in the sediment-derived BES compared with the organic derived fertilizer BES may be associated with differences in the microbial population originating from the respective inocula. Sediments, particularly anaerobic sediments, can harbor microorganisms capable of EET and may therefore provide favorable conditions for the enrichment of electroactive population on the anode. Under the same isolated conditions, the sediment inoculum also yielded a broader diversity of culturable bacterial strains than the organic fertilizer inoculum, including several genera previously associated with electrochemical activity in BESs, such as *Aeromonas*, *Pseudomonas*, *Citrobacter*, *Enterobacter*, *Delftia*, and *Comamonas*. For example, *Aeromonas* strains have been reported to perform EET, electroactive *Citrobacter* and *Enterobacter* strains have been isolated from microbial fuel, and *Pseudomonas* species can facilitate EET through redox-active phenazine compounds. Although the recovery of these genera indicates the presence of potentially electroactive microorganisms in the sediment inoculum, it does not demonstrate that these organisms were directly responsible for the higher current output. Previous studies have shown that the inoculum source can strongly influence anodic microbial community composition and BES performance, with sediment derived inocula in some cases exhibiting greater electricity generation than other environmental inocula (Ishii et al., 2017; Mei et al., 2015; Sun et al., 2016). Thus, the higher performance of the sediment-derived BES may reflect differences in the abundance or activity of electroactive microorganisms, overall community composition, biofilm development, or EET capability between the two inocula.

A total of 27 strains were isolated from sodium lactate-enriched BESs

(Table 2), comprising 20 from sediment and 7 from organic fertilizer inoculant. The sediment-derived strains are phylogenetically diverse, representing members of the genera *Pseudomonas*, *Paenacidovorax*, *Simplicispira*, *Paracidovorax*, *Ralstonia*, *Comamonas*, *Cupriavidus*, *Microbacterium*, *Enterobacter*, *Citrobacter*, *Delftia*, and *Aeromonas*. All isolates exhibited high sequence similarity (97.00–100%) with their nearest type strains based on 16S rRNA gene sequence analysis. The phylogenetic relationships of the isolated strains are further illustrated by the individual phylogenetic trees provided in Fig. S2. Several species were represented by multiple isolates, including *P. guezennnei* (AS-14 and AS-27), *C. composti* (AS-15 and AS-16), *C. terrigena* (AS-19 and AS-28), and *D. acidovorans* (AS-23 and AS-24). These results align with earlier studies reporting the enrichment of hydrolytic and lactate-utilizing bacteria under similar conditions (Kumar et al., 2016; Wang et al., 2022).

5.3. Bioelectricity generation performance of monocultures in BESs

In this study, the bioelectricity production performance of diverse isolated microbial strains was evaluated at 60 h in BES. As shown in Fig. 2a and b, the abiotic control showed negligible current generation, with a peak current density of $-0.02 \mu\text{A cm}^{-2}$ and an average current density of $-0.21 \mu\text{A cm}^{-2}$. The results clearly demonstrate substantial variability in current production among the isolates. As indicated in Fig. 2i and j, *A. eucrenophila* AS-26 and *P. citrulli* AS-12 exhibited the highest performance, with peak current densities of 2.18 and 2.10 $\mu\text{A cm}^{-2}$, respectively, and correspondingly high mean values of 1.78 and 1.50 $\mu\text{A cm}^{-2}$, respectively. These values were higher than those of the other isolates. Comparable current outputs have been reported for *Gordonia* sp. RH-1 and *Stutzerimonas* sp. CH-1, with values of $2.19 \pm 0.46 \mu\text{A cm}^{-2}$ and $1.73 \pm 0.47 \mu\text{A cm}^{-2}$, respectively (Mukherjee and Doyle, 2025).

The relatively high electricity generation performance of *A. eucrenophila* AS-26 may be associated with its physiological characteristics and capacity to participate in EET. Electrochemically active *Aeromonas* strains have previously been isolated from microbial fuel cells and shown to contribute to current generation (Chung and Okabe, 2009; Pham et al., 2003), supporting the potential electroactive

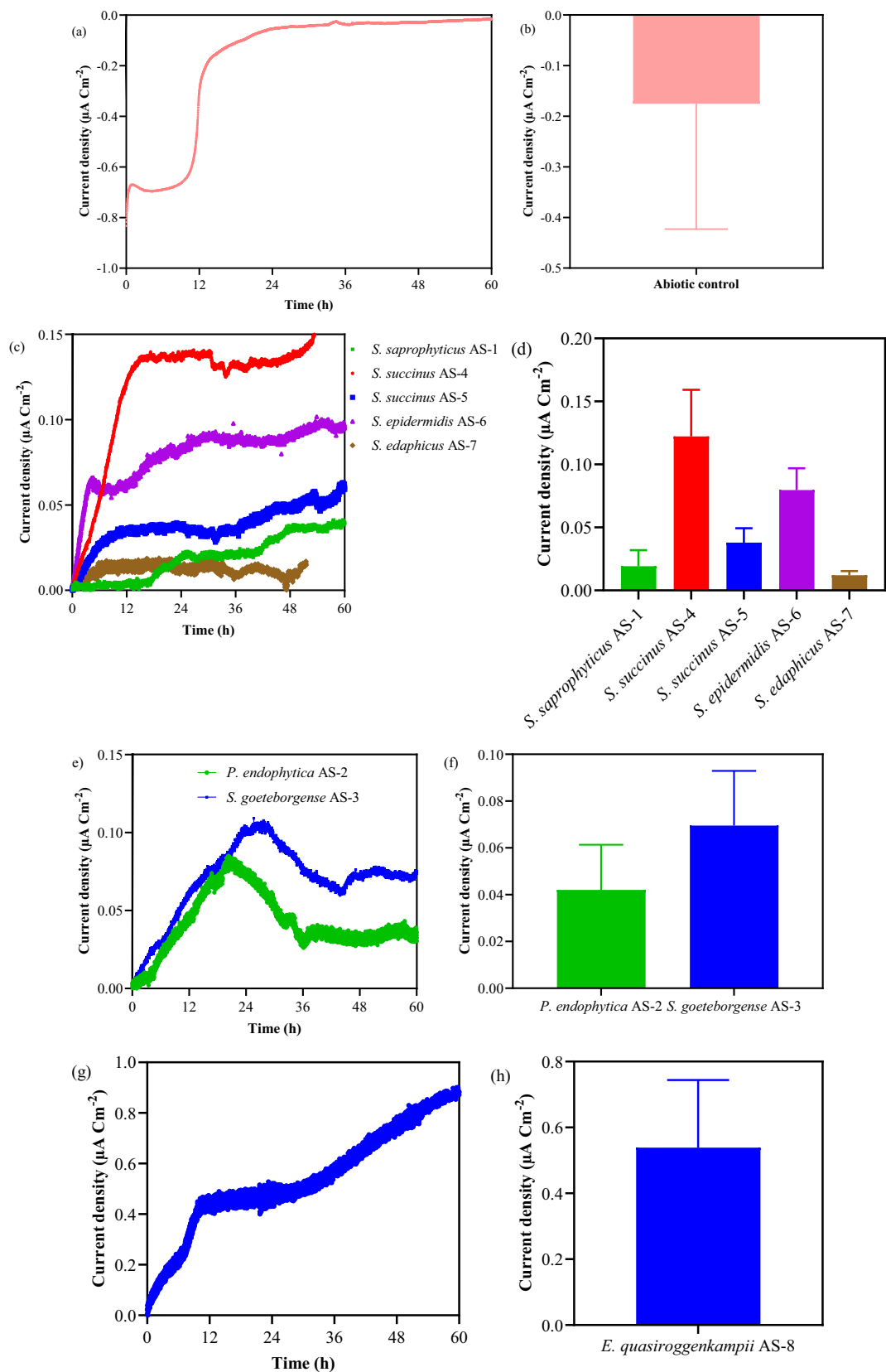


Fig. 2. Bioelectricity generation performance of isolated strains in BESs. Current density profile and average current densities of (a, b) the abiotic control, (c, d) soil derived isolates, (e, f) sediment derived isolates, (g, h) *E. quasiroggenkampii* AS-8 isolated from wastewater, (i, j) sediment-inoculum isolates, and (k,l) organic fertilizer inoculum isolates.

behavior of this genus. In addition, efficient utilization of the supplied electron donor may enhance intracellular electron availability for

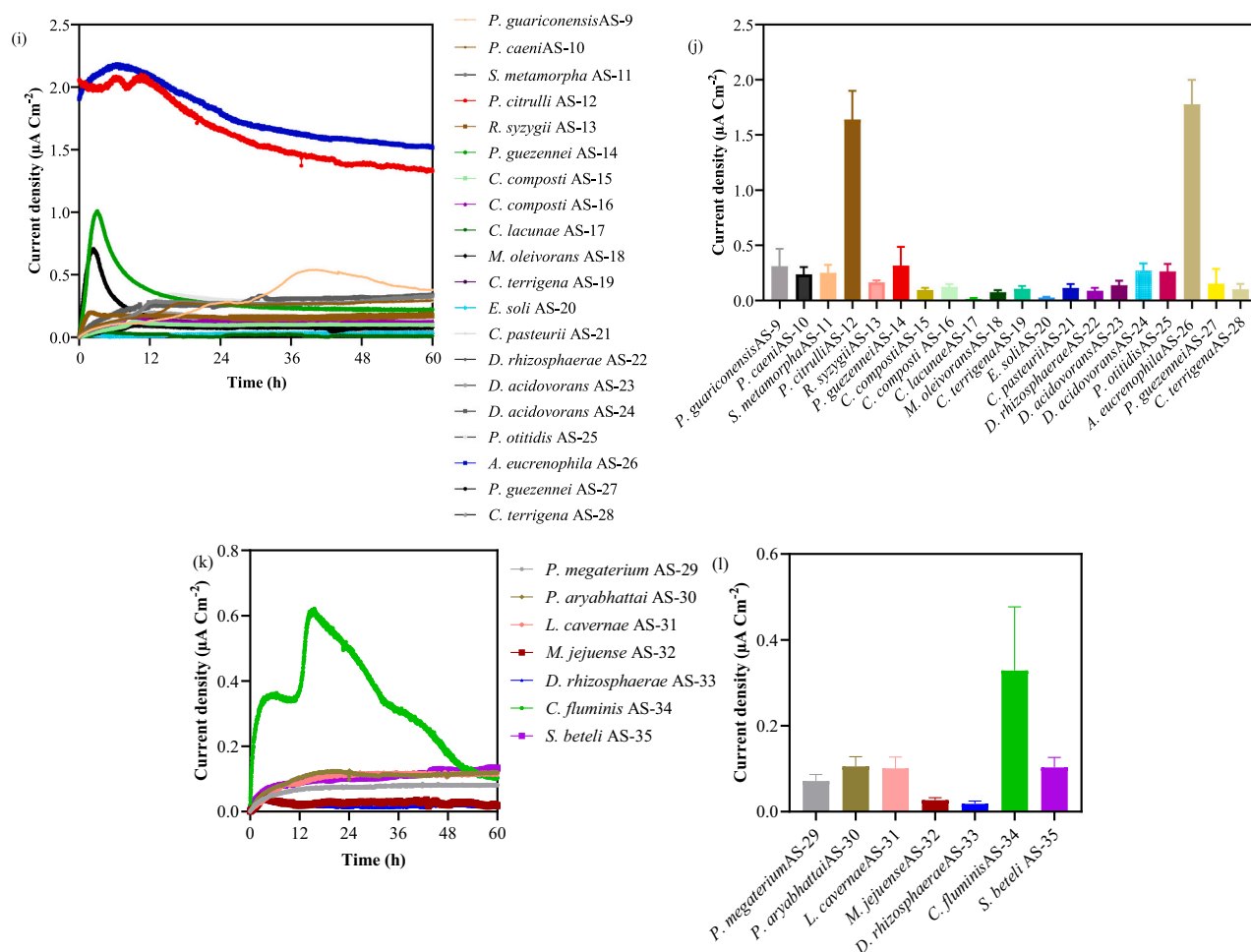
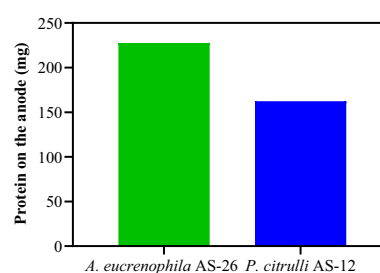


Fig. 2. (continued).

subsequent transfer to extracellular electron acceptors. For example, in the model exoelectrogen *Shewanella oneidensis* MR-1, lactate metabolism generates intracellular reducing equivalents that can subsequently contribute to EET (Ishiki and Shiigi, 2019). Thus, differences in lactate utilization, metabolic activity, biofilm formation, and cell-electrode interactions may partly account for the relatively high-electricity-generation performance of *A. eucorenophila* AS-26 and *P. citrulli* AS-12.

In contrast, several isolates exhibited low current production. For example, environmental sediment-derived isolates, *P. endophytica* AS-2 and *S. goeteborgense* AS-3 showed low current densities, with peak values of 0.08 and 0.11 $\mu\text{A cm}^{-2}$ and average values of 0.04 and 0.07 $\mu\text{A cm}^{-2}$, respectively (Fig. 2e and f). Similarly, *S. edaphicus* AS-7 and *C. lacunae* AS-17 showed negligible current production, with peak current densities of 0.02 and 0.05 $\mu\text{A cm}^{-2}$, respectively (Fig. 2c, d, i and j). Similarly, *Citrobacter freundii* An1 isolated from microbial fuel cell showed negligible bioelectricity generation as a monoculture, with a peak current density below 0.2 $\mu\text{A cm}^{-2}$ (Xiao et al., 2021).

Several genera including *Pseudomonas*, *Enterobacter*, *Comamonas*, and *Delftia* showed moderate bioelectricity generation. *E. quasiroggenkampii* AS-8 produced a peak current density of 0.90 $\mu\text{A cm}^{-2}$ (Fig. 2g and h). *P. guezzennei* AS-14 and *C. fluminis* AS-34 exhibited peak current densities of 1.00 and 0.62 $\mu\text{A cm}^{-2}$, respectively (Fig. 2i and j, and Fig. 2k and l).

Fig. 3. Anode-attached biofilm protein contents of *A. eucorenophila* AS-26 and *P. citrulli* AS-12.

5.4. Quantification of anode-attached biomass and biofilm visualization of selected strains

5.4.1. Quantification of anode-attached biomass in selected strains using the BCA assay

To further assess microbial biomass on the electrode surface, anode associated protein content was quantified using the BCA assay after pretreatment of the electrode in 0.1 M NaOH at 90 °C for 15 min. A standard calibration curve was established as ($Y = 0.0009x + 0.0979$), with R^2 value of 0.9995. The protein contents of the anodes colonized by *A. eucorenophila* AS-26 and *P. citrulli* AS-12 were subsequently calculated from the calibration curve as shown in Fig. 3. The anode associated protein content of *A. eucorenophila* AS-26 was 227.59 mg, whereas that of *P. citrulli* AS-12 was 162.41 mg.

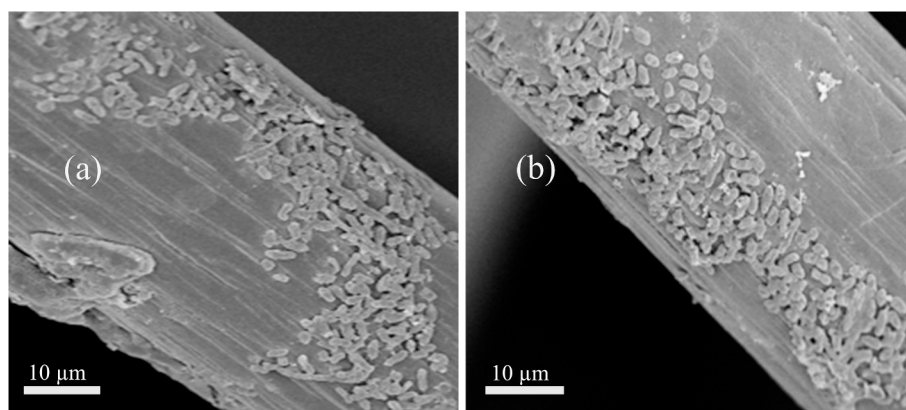


Fig. 4. Anode-attached biofilm visualization by scanning electron microscopy. (a) *A. eucrenophila* AS-26. (b) *P. citrulli* AS-12.

Anode-associated microbial biomass is an important factor affecting bioelectricity generation because the formation of biofilms promotes microbial attachment and facilitate electron transfer to the anode. Previous studies have shown that increased active biomass on the anode improve microbial fuel cell performance (Li et al., 2017). A positive relationship between viable anode biofilm cells and power generation has also been reported (Ren et al., 2011). In addition, higher anode biomass has been associated with increased current generation under suitable operating conditions (Li et al., 2016).

5.4.2. Anode-attached biofilm visualization of selected strains by SEM

SEM analysis was performed to examine bacterial attachment and biofilm formation on the anode surface. As shown in Fig. 4a and b, numerous bacterial cells were attached to the carbon felt surfaces colonized by *A. eucrenophila* AS-26 and *P. citrulli* AS-12. The cells were distributed in clusters along the electrode surface, indicating effective colonization and biofilm formation by both isolates. Biofilm formation provides close contact between microorganisms and the electrode surface which can facilitate EET and contribute to bioelectricity generation (Saratale et al., 2017).

5.5. Study limitations and future perspectives

The present study mainly evaluates the bioelectricity generation performance of the isolated strains based on the current density. Detailed electrochemical characterization, including cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and polarization curves was not performed. These analyses could provide further information on redox activity, electron transfer resistance, electrode interaction, and overall, the electrochemical performance. Future studies will therefore include these electrochemical measurements to better understand the EET characteristics of the isolated strains. In addition, the microbial strains identified in this study provide candidates for future coculture studies with *S. oneidensis* MR-1 and other microorganism using lactate as a carbon source, to investigate microbial interactions and their effects on bioelectricity generation.

6. Conclusions

This study demonstrates the utility of sodium lactate-based enrichment for recovering environmentally derived microbial strains with potential bioelectrochemical activity. Through sodium lactate enrichment, diverse microbial strains were isolated from soil, sediment, wastewater, and BES, and their bioelectricity performance was evaluated. These findings highlight the effectiveness of substrate-driven enrichment strategies for obtaining microbial strains with potential application in bioelectrochemical systems.

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CRediT authorship contribution statement

Asmamaw Abat Getu: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Md Sourav Sarker:** Writing – review & editing. **Juvenis Sugira Murekezi:** Writing – review & editing. **Geng Chen:** Writing – review & editing, Resources. **Yong Xiao:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (Version 5.1 and 5.6, OpenAI) to improve spelling, grammar and to assist with figure modification and generation. After using this tool, the authors reviewed and edited the contents as needed and take full responsibility for the content of the published article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

All the data analyzed and presented in this study are available from the corresponding author upon request.

References

- Aiyer, K.S., 2021. Synergistic effects in a microbial fuel cell between co-cultures and a photosynthetic alga *Chlorella vulgaris* improve performance. *Heliyon* 7, e05935. <https://doi.org/10.1016/j.heliyon.2021.e05935>.
- Beecroft, N.J., Zhao, F., Varcoe, J.R., Slade, R.C., Thumser, A.E., Avignone-Rossa, C., 2012. Dynamic changes in the microbial community composition in microbial fuel cells fed with sucrose. *Appl. Microbiol. Biotechnol.* 93, 423–437. <https://doi.org/10.1007/s00253-011-3590-y>.
- Cao, Y., Mu, H., Liu, W., Zhang, R., Guo, J., Xian, M., Liu, H., 2019. Electricigens in the anode of microbial fuel cells: pure cultures versus mixed communities. *Microb. Cell Factories* 18, 39. <https://doi.org/10.1186/s12934-019-1087-z>.
- Cao, L., Sun, H., Ma, Y., Lu, M., Zhao, M., Li, E., Liu, Y., 2023. Analysis and enhancement of the energy utilization efficiency of corn Stover using strain Lsc-8 in a bioelectrochemical system. *Microb. Cell Factories* 22, 54. <https://doi.org/10.1186/s12934-023-02058-6>.
- Christgen, B., Spurr, M., Milner, E.M., Izadi, P., McCann, C., Yu, E., Curtis, T., Scott, K., Head, I.M., 2023. Does pre-enrichment of anodes with acetate to select for *Geobacter* spp. enhance performance of microbial fuel cells when switched to more complex substrates? *Front. Microbiol.* 14, 1199286. <https://doi.org/10.3389/fmicb.2023.1199286>.
- Chung, K., Okabe, S., 2009. Characterization of electrochemical activity of a strain ISO2-3 phylogenetically related to *Aeromonas* sp. isolated from a glucose-fed microbial fuel cell. *Biotechnol. Bioeng.* 104, 901–910. <https://doi.org/10.1002/bit.22453>.
- Dantanarayana, A., Beaver, K., Van Devenner, B., Chandler, N.B., Minter, S.D., 2025. Understanding palladium ion induced bioelectrocatalysis in *Shewanella oneidensis* MR-1 through electrochemical and genetic interrogations. *ACS Electrochem.* 1, 2198–2208. <https://doi.org/10.1021/acselectrochem.5c00244>.
- De La Cruz-Noriega, M., Benites, S.M., Rojas-Flores, S., Otiniano, N.M., Sabogal Vargas, A.M., Alfaro, R., Cabanillas-Chirinos, L., Rojas-Villacorta, W., Nazario-Naveda, R., Delfín-Narciso, D., 2023. Use of wastewater and electrogenic bacteria to generate eco-friendly electricity through microbial fuel cells. *Sustainability* 15, 10640. <https://doi.org/10.3390/su151310640>.
- Fayad, L., Amer, R., Abd El Nabi, A., Hammoud, A., Yusef, H., 2022. Isolation and characterization of cellulose degrading microorganisms generating electricity using microbial fuel cell. *BAU J. Sci. Technol.* 4, 5. <https://doi.org/10.54729/ROGR4946>.
- Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791. <https://doi.org/10.2307/2408678>.
- Hamzah, A.F., Abd-alsahib, W.H., Mahdi, S.S., 2020. Isolation and identification new bacterial strains isolated from different sources of Al-Rafidiah oil field in Iraq. *Catrina Int. J. Environ. Sci.* 21, 15–22. <https://doi.org/10.21608/cat.2020.23299.1041>.
- Han, J.-Y., Zhang, H.-L., Guo, H., Liu, A.-Q., Nawab, S., Liu, N., Hui, M., Zhai, D.-D., Yong, Y.-C., 2024. A rational designed synthetic three-species alliance system for synergistic improvement on power generation from microbial fuel cell. *Chem. Eng. J.* 481, 148366. <https://doi.org/10.1016/j.cej.2023.148366>.
- Hu, J., Yang, W., Li, L., Liu, Y., 2022. Effects of culture conditions on denitrification activity by *Magnetospirillum gryphiswaldense* strain MSR-1. *J. Water Process Eng.* 50, 103316. <https://doi.org/10.1016/j.jwpe.2022.103316>.
- Huang, J., Sun, B., Zhang, X., 2010. Electricity generation at high ionic strength in microbial fuel cell by a newly isolated *Shewanella marisflavi* EP1. *Appl. Microbiol. Biotechnol.* 85, 1141–1149. <https://doi.org/10.1007/s00253-009-2259-2>.
- Ishii, S.I., Suzuki, S., Yamanaka, Y., Wu, A., Nealon, K.H., Bretschger, O., 2017. Population dynamics of electrogenic microbial communities in microbial fuel cells started with three different inoculum sources. *Bioelectrochemistry* 117, 74–82. <https://doi.org/10.1016/j.bioelechem.2017.06.003>.
- Ishiki, K., Shiigi, H., 2019. Kinetics of intracellular electron generation in *Shewanella oneidensis* MR-1. *Anal. Chem.* 91, 14401–14406. <https://doi.org/10.1021/acs.analchem.9b02900>.
- Jalal, K., Md. Zahangir Alam, M.Z.A., Muyibi, S.A., Jamal, P., 2005. Isolation and purification of bacterial strains from treatment plants for effective and efficient bioconversion of domestic wastewater sludge. *Am. J. Environ. Sci.* <https://doi.org/10.3844/ajessp.2006.1.5>.
- Jiang, Y.-B., Zhong, W.-H., Han, C., Deng, H., 2016. Characterization of electricity generated by soil in microbial fuel cells and the isolation of soil source exoelectrogenic bacteria. *Front. Microbiol.* 7, 1776. <https://doi.org/10.3389/fmicb.2016.01776>.
- Jiang, Z., Zhang, Y., Liu, Z., Ma, Y., Kang, J., Liu, Y., 2018. Isolation and characterization of an exoelectrogenic strain CL-1 from soil and electron transfer mechanism by linking electrochemistry and spectroscopy. *Electrochim. Acta* 292, 982–989. <https://doi.org/10.1016/j.electacta.2018.09.153>.
- Kumar, R., Singh, L., Zularisam, A.W., 2016. Exoelectrogens: recent advances in molecular drivers involved in extracellular electron transfer and strategies used to improve it for microbial fuel cell applications. *Renew. Sust. Energ. Rev.* 56, 1322–1336. <https://doi.org/10.1016/j.rser.2015.12.029>.
- Li, N., Kakarla, R., Min, B., 2016. Effect of influential factors on microbial growth and the correlation between current generation and biomass in an air cathode microbial fuel cell. *Int. J. Hydrog. Energy* 41, 20606–20614. <https://doi.org/10.1016/j.ijhydene.2016.09.094>.
- Li, J., Li, H., Zheng, J., Zhang, L., Fu, Q., Zhu, X., Liao, Q., 2017. Response of anodic biofilm and the performance of microbial fuel cells to different discharging current densities. *Bioresour. Technol.* 233, 1–6. <https://doi.org/10.1016/j.biortech.2017.02.083>.
- Li, Y., Zhang, J., Chen, Q., Tang, S., Peng, Y., 2025. Role of *mtr* pathway in extracellular electron transfer-driven malathion degradation by *Shewanella oneidensis* MR-1. *Chem. Eng. J.*, 166288 <https://doi.org/10.1016/j.cej.2025.166288>.
- Liu, W., Zhu, W., Yao, W., Mao, S., 2007. Isolation and identification of a lactate-utilizing, butyrate-producing bacterium and its primary metabolic characteristics. *Acta Microbiol. Sin.* 47, 435–440.
- Mei, X., Guo, C., Liu, B., Tang, Y., Xing, D., 2015. Shaping of bacterial community structure in microbial fuel cells by different inocula. *RSC Adv.* 5, 78136–78141. <https://doi.org/10.1039/C5RA16382J>.
- Mishra, A., Saxena, A., Singh, S.P., 2019. Isolation and characterization of microbial strains from refinery effluent to screen their bioremediation potential. *J. Pure Appl. Microbiol.* 13, 2325–2332. <https://doi.org/10.22207/JPAM.13.4.48>.
- Mukherjee, D., Doyle, L.E., 2025. Electrochemical enrichment of a community of weak electricigens and characterisation of three halotolerant electroactive isolates: *Micrococcus* sp. YH-1, *Gordonia* sp. RH-1 and *Stutzerimonas* sp. CH-1. *Electrochim. Acta* 510, 145350. <https://doi.org/10.1016/j.electacta.2024.145350>.
- Mulamattathil, S.G., Bezuidenhout, C., Mbewe, M., Ateba, C.N., 2014. Isolation of environmental bacteria from surface and drinking water in Mafikeng, South Africa, and characterization using their antibiotic resistance profiles. *J. Pathog.* 2014, 371208. <https://doi.org/10.1155/2014/371208>.
- Nazeer, Z., Fernando, E.Y., 2022. A novel growth and isolation medium for exoelectrogenic bacteria. *Enzym. Microb. Technol.* 155, 109995. <https://doi.org/10.1016/j.enzymitec.2022.109995>.
- Neetha, J., Ujwal, P., Kumar, K.G., Chidananda, B., Goveas, L., Sandesh, K., 2021. Aerobic biodegradation and optimization of 2, 4-dichlorophenoxyacetic acid by *E. Hormaechei* subsp. *Xiangfangensis* and assessment of biodegraded metabolite toxicity. *Environ. Technol. Innov.* 24, 102055. <https://doi.org/10.1016/j.eti.2021.102055>.
- Pham, C.A., Jung, S.J., Phung, N.T., Lee, J., Chang, I.S., Kim, B.H., Yi, H., Chun, J., 2003. A novel electrochemically active and Fe (III)-reducing bacterium phylogenetically related to *Aeromonas hydrophila*, isolated from a microbial fuel cell. *FEMS Microbiol. Lett.* 223, 129–134. [https://doi.org/10.1016/S0378-1097\(03\)00354-9](https://doi.org/10.1016/S0378-1097(03)00354-9).
- Powers, M.J., Sanabria-Valentín, E., Bowers, A.A., Shank, E.A., 2015. Inhibition of cell differentiation in *Bacillus subtilis* by *Pseudomonas protegens*. *J. Bacteriol.* 197, 2129–2138. <https://doi.org/10.1128/JB.02535-14>.
- Qiu, S., Wang, L., Zhang, Y., Yu, Y., 2022. Microbial fuel cell-based biosensor for simultaneous test of sodium acetate and glucose in a mixed solution. *Int. J. Environ. Res. Public Health* 19, 12297. <https://doi.org/10.3390/ijerph191912297>.
- Raut, S.S., Sharma, A., Mishra, P.K., Kumar, R., Pal, S., Mishra, A., 2025. Isolation and characterization of novel bacterial strain from sewage sludge and exploring its potential for hydrogen production. *Arch. Microbiol.* 207, 10. <https://doi.org/10.1016/j.ijhydene.2013.10.166>.
- Read, S.T., Dutta, P., Bond, P.L., Keller, J., Rabaey, K., 2010. Initial development and structure of biofilms on microbial fuel cell anodes. *BMC Microbiol.* 10, 98. <https://doi.org/10.1186/1471-2180-10-98>.
- Ren, Z., Ramasamy, R.P., Cloud-Owen, S.R., Yan, H., Mench, M.M., Regan, J.M., 2011. Time-course correlation of biofilm properties and electrochemical performance in single-chamber microbial fuel cells. *Bioresour. Technol.* 102, 416–421. <https://doi.org/10.1016/j.biortech.2010.06.003>.
- Rosenbaum, M.A., Bar, H.Y., Beg, Q.K., Segrè, D., Booth, J., Cotta, M.A., Angenent, L.T., 2011. *Shewanella oneidensis* in a lactate-fed pure-culture and a glucose-fed co-culture with *Lactococcus lactis* with an electrode as electron acceptor. *Bioresour. Technol.* 102, 2623–2628. <https://doi.org/10.1016/j.biortech.2010.10.033>.
- Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>.
- Sankari, D., Khuroo, A., Aarti, C., 2020. Isolation of bacteria from soil sample of Tamil Nadu and their in vitro interaction. *World News Nat. Sci.* 28. <https://doi.org/10.1007/s00284-020-02303-w>.
- Saratale, G.D., Saratale, R.G., Shahid, M.K., Zhen, G., Kumar, G., Shin, H.-S., Choi, Y.-G., Kim, S.-H., 2017. A comprehensive overview on electro-active biofilms, role of exoelectrogens and their microbial niches in microbial fuel cells (MFCs). *Chemosphere* 178, 534–547. <https://doi.org/10.1016/j.chemosphere.2017.03.066>.
- Sarmin, S., Tarek, M., Roopan, S.M., Cheng, C.K., Khan, M.M.R., 2021. Significant improvement of power generation through effective substrate-inoculum interaction mechanism in microbial fuel cell. *J. Power Sources* 484, 229285. <https://doi.org/10.1016/j.jpowsour.2020.229285>.
- Sciarria, T.P., Arioli, S., Gargari, G., Mora, D., Adani, F., 2019. Monitoring microbial communities' dynamics during the start-up of microbial fuel cells by high-throughput screening techniques. *Biotechnol. Rep.* 21, e00310. <https://doi.org/10.1016/j.btre.2019.e00310>.
- Silva, L.C.F., Lima, H.S., Mendes, T.A.d.O., Sartoratto, A., Sousa, M.P., de Souza, R.S., de Paula, S.O., de Oliveira, V.M., Silva, C.C., 2020. Physicochemical characterization of *Pseudomonas stutzeri* UVF5 and analysis of its transcriptome under heterotrophic nitrification/aerobic denitrification pathway induction condition. *Sci. Rep.* 10, 2215. <https://doi.org/10.1038/s41598-020-59279-7>.
- Stratford, J.P., Beecroft, N.J., Slade, R.C., Grüning, A., Avignone-Rossa, C., 2014. Anodic microbial community diversity as a predictor of the power output of microbial fuel cells. *Bioresour. Technol.* 156, 84–91. <https://doi.org/10.1016/j.biortech.2014.01.041>.
- Sun, G., de Sacadura Rodrigues, D., Thygesen, A., Daniel, G., Fernando, D., Meyer, A.S., 2016. Inocula selection in microbial fuel cells based on anodic biofilm abundance of *Geobacter sulfurreducens*. *Chin. J. Chem. Eng.* 24, 379–387. <https://doi.org/10.1016/j.cjche.2015.11.002>.
- Tamura, K., Nei, M., Kumar, S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci.* 101, 11030–11035. <https://doi.org/10.1073/pnas.0404206101>.
- Tang, R., Zhang, B., Dai, L., Wang, Y., Xin, G., Liu, Z., Li, F., Song, H., 2024. Engineering artificial three-species microbial consortium to produce high-power bioelectricity

- from discarded cellulosic biomass of liquor industry. *ACS Sustain. Chem. Eng.* 12, 17992–18003. <https://doi.org/10.1021/acssuschemeng.4c04119>.
- Venkataraman, A., Rosenbaum, M.A., Perkins, S.D., Werner, J.J., Angenent, L.T., 2011. Metabolite-based mutualism between *Pseudomonas aeruginosa* PA14 and *Enterobacter aerogenes* enhances current generation in bioelectrochemical systems. *Energy Environ. Sci.* 4, 4550–4559. <https://doi.org/10.1039/C1EE01377G>.
- Vibha, B., Neelam, G., 2012. Importance of exploration of microbial biodiversity. *Int. Res. J. Biol. Sci.* 1, 78–83.
- Wang, D., Wang, Y., Lv, X., Cai, X., Iqbal, W., Yang, B., Zhou, D., Rensing, C., Mao, Y., 2022. Isolation of anaerobic bromate-reducing bacteria using different carbon sources and transcriptomic insights from *Klebsiella variicola* Glu3. *Front. Microbiol.* 13, 851844. <https://doi.org/10.3389/fmicb.2022.851844>.
- Wu, X., Qiao, Y., Guo, C., Shi, Z., Li, C.M., 2020. Nitrogen doping to atomically match reaction sites in microbial fuel cells. *Commun. Chem.* 3, 68. <https://doi.org/10.1038/s42004-020-0316-z>.
- Xiao, Y., Chen, G., Chen, Z., Bai, R., Zhao, B., Tian, X., Wu, Y., Zhou, X., Zhao, F., 2021. Interspecific competition by non-exoelectrogenic *Citrobacter freundii* An1 boosts bioelectricity generation of exoelectrogenic *Shewanella oneidensis* MR-1. *Biosens. Bioelectron.* 194, 113614. <https://doi.org/10.1016/j.bios.2021.113614>.
- You, L.-X., Liu, L.-D., Xiao, Y., Dai, Y.-F., Chen, B.-L., Jiang, Y.-X., Zhao, F., 2018. Flavins mediate extracellular electron transfer in gram-positive *Bacillus megaterium* strain LLD-1. *Bioelectrochemistry* 119, 196–202. <https://doi.org/10.1016/j.bioelechem.2017.10.005>.
- Yu, Y.-Y., Zhen, S.-H., Chao, S.-L., Wu, J., Cheng, L., Li, S.-W., Xiao, X., Zhou, X., 2022. Electrochemistry of newly isolated gram-positive bacteria *Paenibacillus lautus* with starch as sole carbon source. *Electrochim. Acta* 411, 140068. <https://doi.org/10.1016/j.electacta.2022.140068>.
- Zhang, X., Zhang, H., Wang, C., Chen, Q., Zhao, Y., Zhou, Q., Wu, Z., 2020. Isolation of two iron-reducing facultative anaerobic electricigens and probing the application performance in eutrophication water. *Ann. Microbiol.* 70, 21. <https://doi.org/10.1186/s13213-020-01568-7>.
- Zhang, N., Zhang, Y., Bohu, T., Wu, S., Bai, Z., Zhuang, X., 2022. Nitrogen removal characteristics and constraints of an Alphaproteobacteria with potential for high nitrogen content heterotrophic nitrification-aerobic denitrification. *Microorganisms* 10, 235. <https://doi.org/10.3390/microorganisms10020235>.
- Zhou, L., Tang, T., Deng, D., Wang, Y., Pei, D., 2024. Isolation and electrochemical analysis of a facultative anaerobic electrogenic strain *Klebsiella sp.* SQ-1. *Pol. J. Microbiol.* 73, 143. <https://doi.org/10.33073/pjm-2024-013>.