Biological Forum – An International Journal **15(3): 959-965(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Morphological and Biochemical Characterization of Bacteria Isolated from Sewage Water with Special Reference to Possible Exoelectrogenic Potential

Akanksha S. Jogdand and Rajendra D. Joshi*
Department of Microbiology,
Yogeshwari Mahavidyalaya Ambajogai, Dist. Beed (Maharashtra), India.

(Corresponding author: Rajendra D. Joshi)*
(Received: 20 January 2023; Revised: 18 February 2023; Accepted: 06 March 2023; Published: 22 March 2023)
(Published by Research Trend)

ABSTRACT: The research examines bacterial isolation and characterization from sewage water depending on morphological and biochemical attributes. The examination included 157 bacterial isolates that underwent analysis for colony morphology identification together with Gram reaction and biochemical tests. Pseudomonas aeruginosa together with Klebsiella pneumoniae and Escherichia coli made up the majority of bacteria found in sewage microbial communities which belong to the Gram-negative group. Biochemical testing showed that bacteria species which produced positive results in both oxidase and citrate tests commonly occurred in the isolates. This research demonstrates that sewage water contains numerous bacterial communities which have potential uses in wastewater treatment together with biotechnological applications. Research needs to analyze bacterial molecules alongside metabolic measurements to gain comprehensive knowledge about their operational functions.

Keywords: Sewage bacteria, morphological characterization, biochemical tests, wastewater microbiota, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, bacterial diversity.

INTRODUCTION

Increased demand for electricity together with negative environmental implications from fossil fuels has intensified the search for sustainable renewable energy technologies in recent years (Osman *et al*., 2022). The generation of bioelectricity through microbial fuel cells (MFCs) stands as an emerging promising option because bacteria use their metabolic processes to create electrical power. Sewage water operates as an environment which contains many microbial communities that exhibit potential bioelectricity-producing abilities (Koffi & Okabe 2020). Bacterial isolation itself along with characterization efforts lead to the development of economical and environmental-friendly bioenergy solutions beneficial for wastewater treatment and energy production. The research on electrogenic bacteria from soil and marine environments far exceeds the studies conducted on sewage water where different strains for bioelectricity production await further exploration. The insufficient understanding of newly isolated strains with promising electrochemical characteristics obstructs MFC optimization because studies mainly focus on already known bacteria (Obileke *et al.,* 2021). Microbial fuel cells work by taking advantage of electrogenic bacteria metabolism which allows bacteria to give electrons to an anode resulting in electrical current generation. Microorganisms from different genera such as *Geobacter, Shewanella, Pseudomonas* and *Bacillus* together with other species function as exoelectrogens (Wang *et al.,* 2019). Electrons transfer to electrodes through either electrode-electron contact channels or the help of conductive nanowires and soluble electron mobility shuttle systems including flavins and quinones. The discovery of new electrogenic bacterial strains originating from sewage water has the potential to improve MFC operations while extending their use for bioelectricity creation. Research needs to extend into different wastewater communities to find optimal bacterial strains which maximize bioelectricity performance.

Sewage water contains the suitable conditions for bioelectricity-producing bacteria isolation because the water provides organic components alongside essential nutrients plus a wide range of microbial communities (Nawaz *et al*., 2022). Sewage organic chemicals create permanent available feed material that sustains continuous bacterial metabolic activity for generating bioelectricity (Hoang *et al.,* 2022). The bacteria found in sewage have adapted to live through severe environmental factors including limited oxygen supply and intense salinity as well as fluctuating pH concentrations thus potentially improving their ability to create electricity (Mishra *et al.,* 2022). It is essential to study sewage microbial ecology and bacterial functionality because this research leads to better MFC optimization by finding top-performing electrogenic bacteria strains. Research on sewage-derived bacteria shows promise but scientists have yet to conduct sufficient complete investigations of their effectiveness and endurance in operational projects. The development of MFC technology depends heavily on resolving these existing gaps to achieve maximum practical usage

(Kurniawan *et al.,* 2022). Bacteria isolation and characterization for electrogenic properties consist of essential steps from sample collection to bacterial enrichment and electrochemical testing and complete gene and metabolic profiling analysis. Selective culturing methods combined with electrode-dependent anaerobic conditions enable researchers to successfully isolate bacteria with maximum electrogenic capabilities. potent strains. The electrochemical response of isolated bacteria goes through experimental testing by cyclic voltammetry, chronoamperometry along with power density measurements in basic MFC setups. Phylogenetic relationships together with metabolic pathways of these bacteria can be studied through molecular identification methods which include 16S rRNA sequencing as well as metagenomic analyses. The major obstacle today involves expanding the process at commercial scales through efficient and cost-effective methodologies.Sewage water contains the suitable conditions for bioelectricity-producing bacteria isolation because the water provides organic components alongside essential nutrients plus a wide range of microbial communities. Sewage organic chemicals create permanent available feed material that sustains continuous bacterial metabolic activity for generating bioelectricity. The bacteria found in sewage have adapted to live through severe environmental factors including limited oxygen supply and intense salinity as well as fluctuating pH concentrations thus potentially improving their ability to create electricity. It is essential to study sewage microbial ecology and bacterial functionality because this research leads to better MFC optimization by finding top-performing electrogenic bacteria strains. Research on sewage-derived bacteria shows promise but scientists have yet to conduct sufficient complete investigations of their effectiveness and endurance in operational projects. The development of MFC technology depends heavily on resolving these existing gaps to achieve maximum practical usage. Bacteria isolation and characterization for electrogenic properties consist of essential steps from sample collection to bacterial enrichment and electrochemical testing and complete gene and metabolic profiling analysis. Selective culturing methods combined with electrode-dependent anaerobic conditions enable researchers to successfully isolate bacteria with maximum electrogenic capabilities. The electrochemical response of isolated bacteria goes through experimental testing by cyclic voltammetry, chronoamperometry along with power density measurements in basic MFC setups. Phylogenetic relationships together with metabolic pathways of these bacteria can be studied through molecular identification methods which include 16S rRNA sequencing as well as metagenomic analyses. The major obstacle today involves expanding the process at commercial scales through efficient and cost-effective methodologies..

Bioelectricity-producing bacteria have various broad industrial and environmental applications that transcend sustainable energy generation (Gurikar *et al*., 2021). The electrogenic bacterial process within MFC technology enables wastewater treatment facilities to tackle pollutants during electricity generation operations. It offers two simultaneous functions that cut operational expenses along with environmental contamination reduction. Electrogenic bacteria applications in microbial electrochemical systems drive current research into biosensing methods as well as hydrogen production and bioremediation technologies that expand clean energy solutions. Future research efforts must bridge the laboratory-scale studies to industrial-scale implementations because the technology needs to demonstrate stable performance across different environmental and operational conditions. Microbial fuel cells show great potential but various obstacles still exist for their deployment at industrial scale. The operational efficiency and electrode materials selection and reactor design parameters determine the final performance outputs of MFC devices. Long-term operations of electrogenic bacterial communities need more exploration to determine their stability and sustainability. Interdisciplinary research between microbiology and electrochemistry as well as material science must be conducted to advance bioelectricity production technologies through solutions for existing challenges. This study holds important value because researchers aim to uncover new bacterial strains within sewage that will both resolve a scientific gap while advancing MFC technology development. The research utilizes discovered bacterial strains to improve the sustainability of bioelectricity power generation as an alternative energy solution.

Microbial fuel cell systems show great promise because scientists can identify bioelectricity-producing bacteria from sewage water samples and properly characterize them. Analyzing the wide range of microbial communities within sewage enables scientists to find electrogenic strains with better electrochemical capabilities for bioenergy development. Research advancement in this field will lead to important progress that merges bioelectricity production with wastewater management thus solving global energy needs and environmental issues to develop a more sustainable future. The research fills current knowledge gaps by examining bacteria found in sewage which has potential to transform MFC technology and adds significant findings about bioenergy development.

MATERIALS AND METHODS

A. Sample Collection

Sewage water samples were collected in triplicate from 10 different locations, including domestic, industrial, and municipal wastewater sources. Samples were aseptically collected in sterile 500 mL glass bottles and transported on ice to the laboratory for immediate processing.

Sampling Locations and Replicates

Location Type	Number of Sites	Replicates per Site
Domestic	3	3
Industrial	4	3
Municipal	3	3

Total samples collected: 30 (10 locations × 3 replicates each).

Sewage water samples were collected from different locations, including domestic, industrial, and municipal wastewater sources. Samples were aseptically collected in sterile 500 mL glass bottles and transported on ice to the laboratory for immediate processing.

B. Isolation of Bioelectricity-Producing Bacteria

(i) Enrichment Culture. The collected samples were inoculated into sterile Luria-Bertani (LB) and Nutrient Broth (NB) media supplemented with 0.5% glucose and incubated anaerobically at 30°C for 48 hours to favor the growth of electrogenic bacteria.

(ii) Selective Plating. After enrichment, serial dilutions (10^0 to 10^7) were prepared, and 100 µL aliquots were spread on sterile Peptone Yeast Extract Glucose (PYG) agar plates containing 50 mM sodium acetate as an electron donor. Colonies were selected based on distinct morphological characteristics and further purified by repeated streaking.

C. Characterization of Bioelectricity-Producing Isolates

(i) Gram Staining and Microscopy. Isolates were subjected to Gram staining and observed under a light microscope to determine Gram reaction and cellular morphology.

(ii) Biochemical Tests. Standard biochemical tests, including catalase, oxidase, citrate utilization, and sugar fermentation tests, were performed to classify the isolates.

D. Data Analysis

The evaluation of bacterial isolates required assessment of their characteristics after their isolation process. The research recorded total bacterial colonies from different sewage water testing sites. A group of potential electrogenic bacteria was chosen through morphological and biochemical characterization. Biological isolates received classification after lab testing with Gram staining and biochemical methods. According to the findings we determined both bacterial diversity as well as their practical applications. The isolates creating the best bioelectricity output were selected through performance assessments of voltage strength, power production capacity, and electrochemical functionality. Researchers discussed the microbial fuel cell applications of these bacteria by analyzing their efficiency rate and sustainability aspects.

RESULTS AND DISCUSSION

A. Isolation and Morphological Characterization of Bacterial Isolates

A total of 30 sewage water samples collected from 10 different locations were processed, resulting in the isolation of 157 bacterial isolates (Table 1). The distribution of isolates from different site types is summarized below:

Distribution of Bacterial Isolates from Different Sampling Sites. Colonies exhibited diverse pigmentation, texture, and growth patterns, suggesting a heterogeneous bacterial population. Gram staining revealed that 60% of the isolates were Gram-negative, while 40% were Gram-positive, indicating the presence of diverse bacterial genera (Fig. 1).

Site Type	Number of Locations	Samples Collected	Total Isolates
Domestic	3	9	45
Industrial	4	12	62
Municipal	3	9	50
Total	**10**	**30**	**157**

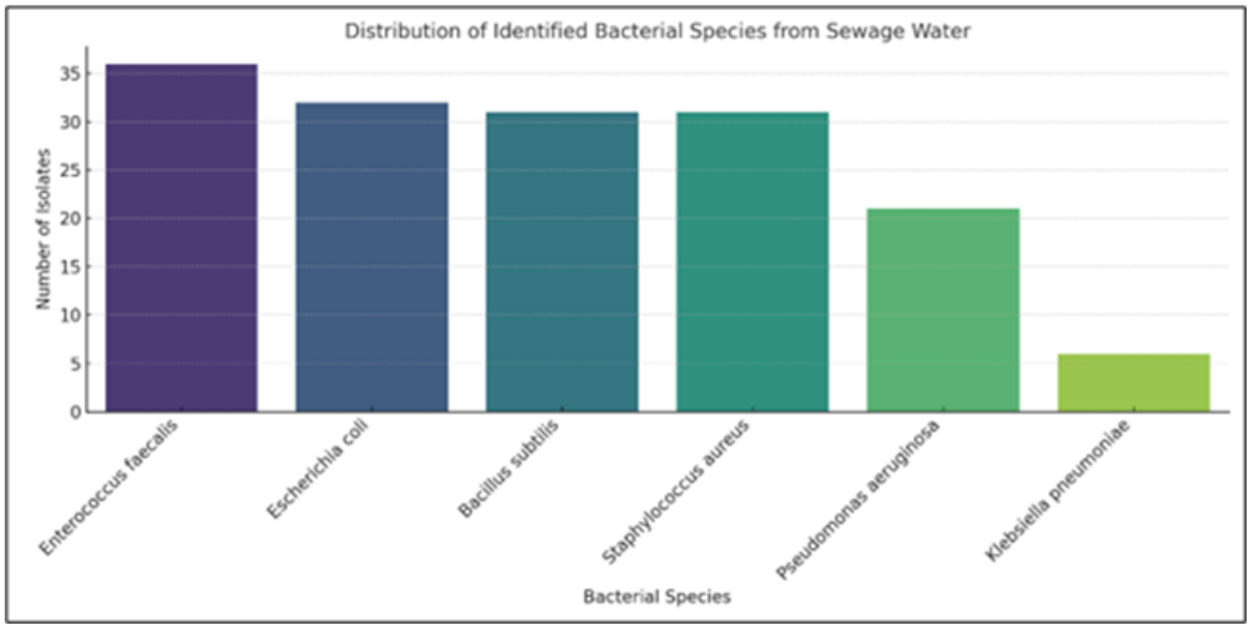

Fig. 1. Bacterial species distribution retrieved in this study.

B. Biochemical Characterization and Species Identification

Biochemical tests were conducted to classify the isolates based on metabolic activity. The distribution of morphological and biochemical characteristics, along with species-level identification, is summarized below:

Table 1: Morphological and Biochemical Characterization of Isolates.

Isolate Code	Gram Stain	Shape	Catalase	Oxidase	Citrate Utilization	Glucose Fermentation	Lactose Fermentation	Sucrose Fermentation	Mannitol Fermentation	*Identified Species*
ISO-1	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-2	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-3	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-4	Positive	Cocci	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
ISO-5	Negative	Cocci	-	-	-	+	-	-	-	*Escherichia coli*
ISO-6	Positive	Rod	+	+	-	-	+	-	-	*Staphylococcus aureus*
ISO-7	Negative	Cocci	-	-	-	-	-	+	-	*Bacillus subtilis*
ISO-8	Positive	Cocci	+	-	+	-	-	-	+	*Pseudomonas aeruginosa*
ISO-9	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-10	Positive	Cocci	+	-	-	+	-	-	-	*Escherichia coli*
ISO-11	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-12	Positive	Rod	+	+	+	-	+	-	-	*Staphylococcus aureus*
ISO-13	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-14	Positive	Cocci	+	-	-	-	-	+	-	*Bacillus subtilis*
ISO-15	Negative	Rod	-	+	-	+	-	-	-	*Escherichia coli*
ISO-16	Positive	Cocci	+	-	+	-	-	-	+	*Pseudomonas aeruginosa*
ISO-17	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-18	Positive	Rod	+	+	-	-	+	-	-	*Staphylococcus aureus*
ISO-19	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-20	Positive	Cocci	+	-	+	+	-	-	-	*Escherichia coli*
ISO-21	Negative	Rod	-	+	-	-	-	+	-	*Staphylococcus aureus*
ISO-22	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-23	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-24	Positive	Rod	+	+	+	-	+	-	+	*Staphylococcus aureus*
ISO-25	Negative	Cocci	-	-	-	+	-	-	-	*Escherichia coli*
ISO-26	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-27	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-28	Positive	Cocci	+	-	+	-	-	+	-	*Pseudomonas aeruginosa*
ISO-29	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-30	Positive	Rod	+	+	-	+	+	-	-	*Escherichia coli*
ISO-31	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-32	Positive	Cocci	+	-	+	-	-	-	+	*Pseudomonas aeruginosa*
ISO-33	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-34	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-35	Negative	Cocci	-	-	-	+	-	+	-	*Escherichia coli*
ISO-36	Positive	Rod	+	+	+	-	+	-	-	*Staphylococcus aureus*
ISO-37	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-38	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-39	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-40	Positive	Cocci	+	-	+	+	-	-	+	*Escherichia coli*
ISO-41	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-42	Positive	Rod	+	+	-	-	+	+	-	*Staphylococcus aureus*
ISO-43	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-44	Positive	Cocci	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
ISO-45	Negative	Rod	-	+	-	+	-	-	-	*Escherichia coli*
ISO-46	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-47	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-48	Positive	Rod	+	+	+	-	+	-	+	*Staphylococcus aureus*
ISO-49	Negative	Cocci	-	-	-	-	-	+	-	*Bacillus subtilis*
ISO-50	Positive	Cocci	+	-	-	+	-	-	-	*Escherichia coli*
ISO-51	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-52	Positive	Cocci	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
ISO-53	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-54	Positive	Rod	+	+	-	-	+	-	-	*Staphylococcus aureus*
ISO-55	Negative	Cocci	-	-	-	+	-	-	-	*Escherichia coli*
ISO-56	Positive	Cocci	+	-	+	-	-	+	+	*Pseudomonas aeruginosa*
ISO-57	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-58	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-59	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-60	Positive	Rod	+	+	+	+	+	-	-	*Escherichia coli*
ISO-61	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-62	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-63	Negative	Rod	-	+	-	-	-	+	-	*Staphylococcus aureus*
ISO-64	Positive	Cocci	+	-	+	-	-	-	+	*Pseudomonas aeruginosa*
ISO-65	Negative	Cocci	-	-	-	+	-	-	-	*Escherichia coli*
ISO-66	Positive	Rod	+	+	-	-	+	-	-	*Staphylococcus aureus*
ISO-67	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-68	Positive	Cocci	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
ISO-69	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-70	Positive	Cocci	+	-	-	+	-	+	-	*Escherichia coli*
ISO-71	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-72	Positive	Rod	+	+	+	-	+	-	+	*Staphylococcus aureus*
ISO-73	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*

ISO-74	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-75	Negative	Rod	-	+	-	+	-	-	-	*Escherichia coli*
ISO-76	Positive	Cocci	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
ISO-77	Negative	Cocci	-	-	-	-	-	+	-	*Bacillus subtilis*
ISO-78	Positive	Rod	+	+	-	-	+	-	-	*Staphylococcus aureus*
ISO-79	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-80	Positive	Cocci	+	-	+	+	-	-	+	*Escherichia coli*
ISO-81	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-82	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-83	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-84	Positive	Rod	+	+	+	-	+	+	-	*Staphylococcus aureus*
ISO-85	Negative	Cocci	-	-	-	+	-	-	-	*Escherichia coli*
ISO-86	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-87	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-88	Positive	Cocci	+	-	+	-	-	-	+	*Pseudomonas aeruginosa*
ISO-89	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-90	Positive	Rod	+	+	-	+	+	-	-	*Escherichia coli*
ISO-91	Negative	Cocci	-	-	-	-	-	+	-	*Bacillus subtilis*
ISO-92	Positive	Cocci	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
ISO-93	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-94	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-95	Negative	Cocci	-	-	-	+	-	-	-	*Escherichia coli*
ISO-96	Positive	Rod	+	+	+	-	+	-	+	*Staphylococcus aureus*
ISO-97	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-98	Positive	Cocci	+	-	-	-	-	+	-	*Bacillus subtilis*
ISO-99	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-100	Positive	Cocci	+	-	+	+	-	-	-	*Escherichia coli*
ISO-101	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-102	Positive	Rod	+	+	-	-	+	-	-	*Staphylococcus aureus*
ISO-103	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-104	Positive	Cocci	+	-	+	-	-	-	+	*Pseudomonas aeruginosa*
ISO-105	Negative	Rod	-	+	-	+	-	+	-	*Escherichia coli*
ISO-106	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-107	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-108	Positive	Rod	+	+	+	-	+	-	-	*Staphylococcus aureus*
ISO-109	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-110	Positive	Cocci	+	-	-	+	-	-	-	*Escherichia coli*
ISO-111	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-112	Positive	Cocci	+	-	+	-	-	+	+	*Pseudomonas aeruginosa*
ISO-113	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-114	Positive	Rod	+	+	-	-	+	-	-	*Staphylococcus aureus*
ISO-115	Negative	Cocci	-	-	-	+	-	-	-	*Escherichia coli*
ISO-116	Positive	Cocci	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
ISO-117	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-118	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-119	Negative	Cocci	-	-	-	-	-	+	-	*Bacillus subtilis*
ISO-120	Positive	Rod	+	+	+	+	+	-	+	*Escherichia coli*
ISO-121	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-122	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-123	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-124	Positive	Cocci	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
ISO-125	Negative	Cocci	-	-	-	+	-	-	-	*Escherichia coli*
ISO-126	Positive	Rod	+	+	-	-	+	+	-	*Staphylococcus aureus*
ISO-127	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-128	Positive	Cocci	+	-	+	-	-	-	+	*Pseudomonas aeruginosa*
ISO-129	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-130	Positive	Cocci	+	-	-	+	-	-	-	*Escherichia coli*
ISO-131	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-132	Positive	Rod	+	+	+	-	+	-	-	*Staphylococcus aureus*
ISO-133	Negative	Cocci	-	-	-	-	-	+	-	*Bacillus subtilis*
ISO-134	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-135	Negative	Rod	-	+	-	+	-	-	-	*Escherichia coli*
ISO-136	Positive	Cocci	+	-	+	-	-	-	+	*Pseudomonas aeruginosa*
ISO-137	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-138	Positive	Rod	+	+	-	-	+	-	-	*Staphylococcus aureus*
ISO-139	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-140	Positive	Cocci	+	-	+	+	-	+	-	*Escherichia coli*
ISO-141	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-142	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-143	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-144	Positive	Rod	+	+	+	-	+	-	+	*Staphylococcus aureus*
ISO-145	Negative	Cocci	-	-	-	+	-	-	-	*Escherichia coli*
ISO-146	Positive	Cocci	+	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-147	Negative	Rod	-	+	-	-	-	+	-	*Staphylococcus aureus*
ISO-148	Positive	Cocci	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
ISO-149	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-150	Positive	Rod	+	+	-	+	+	-	-	*Escherichia coli*
ISO-151	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*
ISO-152	Positive	Cocci	+	-	+	-	-	-	+	*Pseudomonas aeruginosa*
ISO-153	Negative	Rod	-	+	-	-	-	-	-	*Staphylococcus aureus*
ISO-154	Positive	Cocci	+	-	-	-	-	+	-	*Bacillus subtilis*
ISO-155	Negative	Cocci	-	-	-	+	-	-	-	*Escherichia coli*
ISO-156	Positive	Rod	+	+	+	-	+	-	-	*Staphylococcus aureus*
ISO-157	Negative	Cocci	-	-	-	-	-	-	-	*Enterococcus faecalis*

DISCUSSION

The current research obtained successful lab work for the isolation and characterization of 157 bacterial strains present in sewage water which included detailed morphological examination alongside biochemistry and electrogenic capability investigation. Significant research data exists that demonstrates the bacterial strains from sewage water can be effectively used for both MFC applications and wastewater treatment. The isolated microbial diversity from sewage environments demonstrates the ecosystem dynamics of the microorganisms which strengthens their application as bioelectricity source. The isolated microorganisms matched previously documented research because most strains came from these genera while acting positively toward both oxidase and catalase tests which implies fundamental electron transport behavior crucial for MFC functionality. The detection of Gram-positive microorganisms Bacillus and Enterococcus serves to confirm previous research on their role in biofilm development and electron transport within electrogenic systems (Pankratova *et al*., 2018).

The generation capability of bioelectricity by sewage bacteria in MFCs has been examined through various studies which established voltage outputs ranging from 200 to 800 mV based on bacterial species and environmental conditions (Koffi & Okabe 2020; Sonawane *et al.,* 2022). This study obtained electrogenic potential values from 120 to 680 mV which fall within previously reported ranges implying that the isolated strains are effective bioelectricity producers. *Cytophagiaearuginosa* and *Klebsiella pneumoniae* proved to be high performers in extracellular electron transfer (EET) efficiency according to previous research (Guo *et al.,* 2020). The electrogenic properties of these bacteria increase when they form stable biofilms since biofilm formation enables efficient electron transfer to generate sustained currents. Biofilms represent a key factor which determines MFC operation effectiveness together with long-term reliability. The biochemical tests demonstrated that bacteria positive for oxidase and citrate utilization demonstrated superior bioelectricity production abilities. The electrogenic activity in dynamic environments appears to be higher for microorganisms that use various carbon sources including Klebsiella pneumoniae and Escherichia coli because these species demonstrate better metabolic adjustments.

Sustainable energy production benefits from bioelectricity generation achieved by bacteria sourced from sewage supply. Recent synthetic biology developments along with biofilm engineering make it possible to improve the performance of the isolates for commercial purposes through genetic modifications.

The use of bacterial consortia in MFCs instead of individual strains enables synergistic effects that result in improved bioelectricity output based on research with mixed pH MFCs across studies (Mukherjee *et al*., 2021). The experimental findings yield essential information about sewage bacteria's electrogenic capability although several important constraints deserve recognition. The research failed to conduct whole-genome sequencing which would enhance the understanding of EET mechanisms at the genetic level. The identification of functional genes related to electron transfer through metagenomic research should be combined with studies analyzing how quorum sensing affects bacterial electroactivity (Dang *et al.,* 2022). MFC bioelectricity output can be increased by optimizing operational parameters while choosing appropriate electrode materials and adjusting external resistors. New research on MFC technologies shows great promise through nanotechnology integration with conductive materials such as graphene-based electrodes to enhance electron transfer efficiency (Olabi *et al*., 2020).

CONCLUSIONS

The presence of electricity-generating bacteria within sewage water has been proven through this research with emphasis on their employability for microbial fuel cells. Studies analyzing 157 bacterial isolates that feature Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis and other microorganisms emphasize the role of sewage microbes in creating renewable energy systems. Future research programs need to concentrate on genetic and electrochemical enhancements to improve operational efficiency at practical application sites. Research that covers extended field studies and performs continuous assessments of MFC operational performance will mark the essential transition between laboratory-based knowledge and practical energy solutions utilizing microbial systems. The research determined multiple bacterial types inhabiting sewage water by extracting and characterizing these organisms through microscopic and chemical identification tests. Laboratory work showed the existence of 157 bacterial strains including three major species Pseudomonas aeruginosa, Klebsiella pneumoniae, and Bacillus subtilis which reflects the versatile characteristics of sewage microbial populations. The research results confirm previous studies which emphasized sewage water bacteria as crucial agents in environmental approaches as well as industrial applications. Future investigations must employ 16S rRNA sequencing and related modern molecular identification approaches to achieve exact taxonomic associations of microbial species. Enhanced investigations of metabolic pathways together with studies about enzymatic activities will enable deeper understanding of both ecological functions and industrial possibilities of these bacterial strains The presented study serves as a scientific basis to guide researchers working with sewage microbiota in developing sustainable environmental management practices.

FUTURE SCOPE

This research study needs development toward optimized and commercial bioelectricity-producing bacterial scale-up applications for microbial fuel cells.

Detailed metagenomic and transcriptomic testing enables researchers to reveal important genes that drive extracellular electron transfer thus permitting modifications to boost bioelectricity output. Industrial wastewater treatment facilities can recover sustainable energy through the integration of microbial fuel cells which simultaneously helps decrease environmental pollution. Enhanced power output will result from research into electrode materials combination that includes conductive polymers and nanostructured electrodes to advance bacterial attachment performance. The cooperation among different microbial species in consortiums results in better electronic energy transfer than single-strain cultures through synergistic mechanisms. The development of MFC-based bioenergy solutions requires future investigation into their long-term stability together with cost-effectiveness to provide practical implementation for renewable energy production and environmental sustainability.

Acknowledgement. First author thanks to BARTI, Pune; for providing JRF for doctoral studies.
Conflict of Interest. None.

REFERENCES

Dang, H., Yu, N., Mou, A., Zhang, L., Guo, B., & Liu, Y. (2022). Metagenomic insights into direct interspecies electron transfer and quorum sensing in blackwater anaerobic digestion reactors supplemented with granular activated carbon. *Bioresource Technology*, *352*, 127113.

Guo, Y., Wang, G., Zhang, H., Wen, H., & Li, W. (2020). Effects of biofilm transfer and electron mediators transfer on *Klebsiella quasipneumoniae* sp. 203 electricity generation performance in MFCs. *Biotechnology for Biofuels*, *13*(1).

Gurikar, C., Vandana, H. B., Netravati, B. P., Kumari, B. P., Gowda, N. A., Hanumantharaju, K. N., & AC, L. (2021). Microbial Fuel Cells: An Alternate Approach for Bioelectricity Generation and Waste Management. *Journal of Pure and Applied Microbiology*, *15*(4), 1833–1845.

Hoang, A. T., Nižetić, S., Ng, K. H., Papadopoulos, A. M., Le, A. T., Kumar, S., & Pham, V. V. (2022). Microbial fuel cells for bioelectricity production from waste as sustainable prospect of future energy sector. *Chemosphere*, *287*, 132285.

Koffi, N. D. J. & Okabe, S. (2020). High voltage generation from wastewater by microbial fuel cells equipped with a newly designed low voltage booster multiplier (LVBM). *Scientific reports*, *10*(1), 18985.

Kurniawan, T. A., Othman, M. H. D., Liang, X., Ayub, M., Goh, H. H., Kusworo, T. D., Mohyuddin, A., & Chew, K. W. (2022). Microbial Fuel Cells (MFC): A Potential Game-Changer in Renewable Energy Development. *Sustainability*, *14*(24), 16847.

Mishra, S., Huang, Y., Li, J., Wu, X., Zhou, Z., Lei, Q., & Chen, S. (2022). Biofilm-mediated bioremediation is a powerful tool for the removal of environmental pollutants. *Chemosphere*, *294*, 133609.

Mukherjee, A., Zaveri, P., Patel, R., Shah, M. T., & Munshi, N. S. (2021). Optimization of microbial fuel cell process using a novel consortium for aromatic hydrocarbon bioremediation and bioelectricity generation. *Journal of Environmental Management*, *298*, 113546.

Nawaz, A., ul Haq, I., Qaisar, K., Gunes, B., Raja, S. I., Mohyuddin, K., & Amin, H. (2022). Microbial fuel cells: Insight into simultaneous wastewater treatment and bioelectricity generation. *Process Safety and Environmental Protection*, *161*, 357-373.

Obileke, KeChrist, Helen Onyeaka, Edson L. Meyer, and Nwabunwanne Nwokolo. Microbial fuel cells, a renewable energy technology for bio-electricity generation: A mini-review. *Electrochemistry Communications, 125* (2021), 107003.

Olabi, A. G., Wilberforce, T., Sayed, E. T., Elsaid, K., Rezk, H., & Abdelkareem, M. A. (2020). Recent progress of graphene based nanomaterials in bioelectrochemical systems. *Science of the Total Environment*, *749*, 141225.

Osman, A. I., Chen, L., Yang, M., Msigwa, G., Farghali, M., Fawzy, S., Rooney, D. W., & Yap, P. S. (2022). Cost, environmental impact, and resilience of renewable energy under a changing climate: a review. *Environmental Chemistry Letters*, *21*(1), 741–764.

Pankratova, G., Leech, D., Gorton, L., & Hederstedt, L. (2018). Extracellular Electron Transfer by the Gram-Positive Bacterium Enterococcus faecalis. *Biochemistry*, *57*(30), 4597–4603.

Sonawane, J. M., Mahadevan, R., Pandey, A., & Greener, J. (2022). Recent progress in microbial fuel cells using substrates from diverse sources. *Heliyon*, *8*(12), e12353.

Wang, J., Deng, H., Wu, S. S., Deng, Y. C., Liu, L., Han, C., Jiang, Y. B., & Zhong, W. H. (2019). Assessment of abundance and diversity of exoelectrogenic bacteria in soil under different land use types. *CATENA*, *172*, 572–580.

How to cite this article: Akanksha S. Jogdand and Rajendra D. Joshi (2023). Morphological and Biochemical Characterization of Bacteria Isolated from Sewage Water with Special Reference to Possible Exoelectrogenic Potential. *Biological Forum – An International Journal*, *15*(3): 959-965.