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Characterization of Potential Microbial Fuel Cell (MFC) Producing Bacteria from Mangrove Sediment Substrate

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Abstract: The purpose of this research were to acquire the isolate and to determine the identity of bacteria that potentially as Microbial Fuel Cell (MFC). The bacteria MFC of sediment mangrove isolated by using pour method in Nutrient Agar (NA) media. Characterization isolate did phenotipically based on morphological, biochemical and physiological character. Coloni morphological that observed that are NA media and cell morphological (shape,characteristik Gram and motility). Biochemical characteristic that observed including the use of citrate, gelatin hydrolisis, amylolytic characteristik, cellulolitic characteristtik and carbohidrates fermentation rom various of sugars (sucrose, glucose and lactose). Physiological characteristic includes necessary of oxygen, tolerance of temperature, NaCl and pH. The result of this research obtained as much as 12 bacteria isolates. The bacterial characteristic that obtained that is all strains has shape is steam,Gram positive and charachteristic is motile.

Keywords: Sediment mangrove, Bacteria of MFC, phenotypic characteristics

1. Introduction

MFC is known as a technology that can produce electrical energy through the process of degradation of organic matter by microorganisms through catalytic reactions of bioelectrochemical systems (Alwahab et al., 2024). This method directly converts chemical energy into electrical energy through bioelectrochemical reactions using microorganisms or enzyme catalysts (Islam et al., 2020). MFC can be used in the treatment of both organic and inorganic waste. This process generates electrons as a byproduct and can be utilized as a source of electrical energy. Generally, various forms of organic matter can be used as MFC substrates, such as glucose, starch, fatty acids, amino acids, and proteins, wastewater from humans and animals, as well as organic and inorganic sediments (Sudarman et al., 2020).

Microbes play an important role in the anaerobic bioconversion of a substrate into energy. The microbial group that is often used in MFC systems is the bacterial group

(Shen et al., 2020). The dominant types of bacteria used in MFC systems are *electrigens* and fermentative bacteria groups, but *electrigens* are known to be more effectively used in MFC systems than fermentative bacteria groups (Lovley, 2006). Potential MFC bacteria need to be characterized to know their identity. In general, identification can be done phenotypically, including observations of colony and cell morphology, as well as biochemical and physiological properties (Indriyani et al., 2025).

Bacterial identification can be done by conventional phenotypic characterization through a systematic approach. Bacterial characterization is one of the activities carried out to find out or observe bacteria based on the similarities and differences that each bacterium has. This can be done based on phenotypic traits. The phenotypic properties of bacteria include cytological properties, colony morphological properties, biochemical properties, and physiological properties (Flimban et al., 2019). The utilization of bacteria to produce electrical energy is an effort to develop a more efficient and environmentally friendly energy source.

2. Methods

This research was conducted in the Microbiology Laboratory of the Biology Unit, Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari. The materials used were mangrove sediment, semi-solid NA, NA, NB, 70% alcohol, crystal violet, NaCl, gelatin media, SCA media, CMC media, 1% starch media, glucose, sucrose, lactose, NaOH, lugol, safranin, and *congo red*. MFC bacteria from mangrove sediments were isolated using the pour method on *Nutrient Agar* (NA) media. The selection of isolates is based on the morphological characteristics of colonies in *Nutrient Agar* (NA) media. Characterization of isolates is done phenotypically based on morphological, biochemical, and physiological characters. Colony morphological characters observed were on NA media and cell morphology (shape, Gram properties, and motility). Biochemical characters observed include the use of citrate, gelatin hydrolysis, amylolytic properties, cellulolytic properties, and carbohydrate fermentation of various types of sugar (sucrose, glucose, and lactose). Physiological characters include oxygen demand, temperature tolerance, NaCl tolerance, and pH tolerance.

3. Results and Discussion

Phenotypic characteristics of Microbial Fuel Cell (MFC) bacteria are obtained based on the results of characterization (colonies and cells), physiology, and biochemistry.

3.1. Morpfological Characteritics of Bacterial Isolates

Observation of morphological characters of Microbial Fuel Cell (MFC) bacteria is obtained based on the characterization of colony morphology and cell morphology.

3.1.1. Colony Morphology of Bacterial Isolates

The colony morphology character of the MFC bacterial isolate is based on observation on the nutrient agar (NA) media with an incubation time of 24 hours. Colony morphology characters of bacterial isolates observed include color, shape, edge, elevation, and inner structure, as listed in Tables 1, 2, and 3.

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Table 1. Colony Morphology of MFC Bacterial Isolates of Bungkutoko Mangrove Sediment

No.	Isolate Code	Morphological Character				
		Colony Shape	**Elevation**	**Edge**	**Inner Structure**	**Color**
1	BMS.I1	*Rhizoid*	*Raised*	*Crenate*	*Opaque*	White
2	BMS.I2	*Curled*	*Raised*	*Undulate*	*Opaque*	White
3	BMS.I3	*Amoeboid*	*Raised*	*Crenate*	*Opaque*	White
4	BMS.I4	*Circulair*	*Raised*	*Crenate*	*Opaque*	White
5	BMS.II1	*Curled*	*Raised*	*Lobate*	*Opaque*	White
6	BMS.II2	*Circulair*	*Convex*	*Entire*	*Transaparant*	Cream
7	BMS.III1	*Toruloid*	*Raised*	*Lobate*	*Opaque*	Cream

Description : BMS : Bungkutoko Mangrove Sediment

Table 2. Colony Morphology of MFC Bacterial Isolates of Rawa Aopa Mangrove Sediment

No.	Isolate Code	Morphological Character				
		Colony Shape	**Elevation**	**Colony Shape**	**Inner Structure**	**Color**
1	RAMS.I1	*Toruloid*	*Raised*	*Lobate*	*Opaque*	Cream
2	RAMS.II1	*Circulair*	*low convex*	*Entire*	*Opaque*	White
3	RAMS.III1	*Rhizoid*	*Raised with concave*	*Lobete*	*Opaque*	White
4	SMRA.III2	*Filamentous*	*Raised with concave*	*Undulate*	*Opaque*	White

Description : RAMS : Rawa Aopa Mangrove Sediment

Table 3. Colony Morphology of MFC Bacterial Isolates of Jembatan Triping Mangrove Sediment

No.	Isolate Code	Morphological Character				
		Colony Shape	**Elevasi**	**Tepi**	**Inner Structure**	**Color**
1	JTMS.II1	*Myceloid*	*Low convez*	*Ciliate*	*Opaque*	Yellow
2	JTMS.II2	*Circulair*	*Raised*	*Entire*	*Opaque*	Cream

Description : JTMS: Jembatan Triping Mangrove Sediment

The results of morphological observations of colonies of potential bacterial isolates from MFC mangrove sediments, Bungkutoko, there are seven isolates, Rawa Aopa, there are four isolates, and Triping Bridge, two isolates. Characterization of bacterial colonies cannot be used as a full reference in identifying bacteria, considering that the shape of bacterial colonies of one type is not always the same, as stated by Tasaki et al., (2017), that colony morphology can be influenced by microbial strains and environmental conditions. Different species of microorganisms will form different colony morphologies in the same

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environment, even when we incubate only one microbial strain, different types of colonies appear in response to environmental variations.

3.1.2 Cell Morpholgy of Bacterial Isolates

Cell morphology characteristics of potential MFC bacterial isolates observed include shape, Gram properties, and motility. The cell shape and Gram properties were observed using Nutrient Broth (NB) media, while for motility, semi-solid Nutrient Agar (NA) media was used with an incubation time of 24 hours. The morphological characters of bacterial isolates are listed in Tables 4, 5, and 6.

Table 4. Cell Morphology Character of Potential MFC Bacterial Isolates of Mangrove Sediment of Bungkutoko

No.	Isolate Code	Cell Shape	Gram Characteristic	Motility
1	BMS.I1	Stem	Positive	Motile
2	BMS.I2	Stem	Positive	Motile
3	BMS.I3	Stem	Positive	Motile
4	BMS.I4	Stem	Positive	Motile
5	BMS.II1	Stem	Positive	Motile
6	BMS.II2	Stem	Positive	Motile
7	BMS.III1	Stem	Positive	Motile

Table 5. Cell Morphology Character of Potential MFC Bacterial Isolates of Mangrove Sediment of Rawa Aopa

No.	Isolate Code	Cell Shape	Gram Characteristic	Motility
1	RAMS.I1	Stem	Positive	Motile
2	RAMS.II1	Stem	Positive	Motile
3	RAMS.III1	Stem	Positive	Motile
4	RAMS.III2	Stem	Positive	Motile

Table 6. Cell Morphology Character of Potential MFC Bacterial Isolates of Mangrove Sediment of Rawa Aopa

No.	Isolate Code	Cell Shape	Gram Characteristic	Motility
1	JTMS.II1	Stem	Positive	Motile
2	JTMS.II2	Stem	Positive	Motile

Based on the observation of cell morphology (Tables 4, 5, and 6), it shows that all potential MFC bacterial isolates have bacilli (rod) cell characteristics, Gram-positive properties, and are motile (moving). The rod form in bacteria is more commonly found in water because it is related to material, nutrients, and the form of molecules or ions that are transported from the environment through the membrane with some specific mechanisms into bacterial cells (Bazina et al., 2023).

A motility test is a test that determines the character of bacterial cells and indicates their ability to move. Motility test listed in Tables 4, 5, and 6 showed that 12 isolates of potential MFC bacteria were motile, characterized by the presence of traces of bacterial

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movement on semi-solid NA media. According to Palma et al., (2022), a positive test is characterized by bacterial growth that spreads, then the bacteria move (motile), and if the bacterial growth does not spread, then the bacteria do not move (non-motile). Motile bacteria are generally found in bacilli (rod-shaped) bacteria with the ability to move (motile) because they have a flagellum. Flagellum functions as a means of motion; besides that, it can also be used as an attachment tool (Madigan et al., 2006).

3.2 Biochemical Characters of Bacterial Isolates

Biochemical characteristics of potential MFC bacterial isolates include the catalase test, citrate test, cellulolytic test, gelatin hydrolysis, amylolytic test, and sugar fermentation. Biochemical characters of bacterial isolates are listed in Tables 7, 8, and 9.

Table 7. Biochemical Character of Potential MFC Bacteria of Bungkutoko Mangrove Sediment

No	Isolate Code	Catalase Test	Citrate Test	Cellulolitic Test	Gelatin Hydrolysis	Amylololytic Test	Sugar Fermentation (Gas/ Color Change)		
							Glucose	Sucrose	Lactose
1	BMS.I1	+	-	-	+	+	-/+	-/+	-/+
2	BMS.I2	+	-	-	+	-	-/+	-/+	-/+
3	BMS.I3	+	-	-	+	+	-/+	-/+	-/+
4	BMS.I4	+	-	-	+	+	-/+	-/+	-/+
5	BMS.II1	+	-	-	+	+	-/+	-/+	-/+
6	BMS.II2	+	-	-	+	+	-/-	-/-	-/+
7	BMS.III1	+	-	-	+	+	-/+	-/+	-/+

Description : + : Some Ability; - : no ability
+/+ : Gas/color change
-/- : No Gas/no color change
-/+ : No gas/ color change

Tabel 8. Biochemical Character of Potential MFC Bacteria of Rawa Aopa Mangrove Sediment

No	Isolate Code	Catalase Test	Citrate Test	Cellulolitic Test	Gelatin Hydrolysis	Amylololytic Test	Sugar Fermentation (Gas/ Color Change)		
							Glucose	Sukrosa	Laktosa
1	RAMS.I1	+	-	+	+	+	-/+	-/+	-/+
2	RAMS.II1	+	-	-	+	-	-/+	-/+	-/+
3	RAMS.III1	+	+	+	+	+	-/-	-/-	-/+
4	RAMS.III2	+	-	-	+	+	-/+	-/+	-/+

Description : + : Some ability; - : No abyliti
+/+ : Gas/color change

-/- : No Gas/no color change
-/+ : No gas/ color change

Tabel 9. Biochemical Character of Potential MFC Bacteria of Jembatan Triping Mangrove Sediment

No	Isolate Code	Catalase Test	Citrate Test	Cellulolitic Test	Gelatin Hydrolysis	Amylololytic Test	Sugar Fermentation (Gas/ Color Change)		
							Glucose	Sukrosa	Laktosa
1	JTMS.II1	+	-	+	+	+	-/+	-/+	-/+
2	JTMS.II2	-	-	-	+	+	-/+	-/+	-/+

Description : + : Some ability; - : No ability
+/+ : Gas/color change
-/- : No Gas/no color change
-/+ : No gas/ color change

The results of the catalase test showed that JTMS.II1 isolate was catalase positive and JTMS.II2 isolate was catalase-negative. The positive reaction is characterized by the formation of gas bubbles around the colonies of bacterial isolates that are dripped with 3% H_2O_2, and the negative reaction is characterized by the absence of gas bubbles around the colonies of bacterial isolates after being dripped with 3% H_2O_2. The emergence of air bubbles indicates the formation of oxygen gas from the breakdown of hydrogen peroxide by the bacterial catalase enzyme (Plakar et al., 2025). The results of the citrate test of 2 bacterial isolates showed negative results with no color change. Positive results are characterized by a change in the color of the media from green to blue as an acidic fermentation activity and a change in pH in the test media, given the Brom thymol Blue indicator solution.

The results of the cellulolytic test of 2 bacterial isolates showed negative results for isolate JTMS.II1, namely the absence of a clear zone around the edges of the colony, while isolate JTMS.II2 showed positive results, which were characterized by a clear zone formed around the bacterial colony after adding Congo red and 1 M NaCl. This indicates that isolate JTMS.II2 has the ability to utilize carbon sources contained in CMC media. The cellulolytic test aims to determine the ability of bacterial isolates to produce cellulase enzymes that can break down cellulose into simpler compounds (Dashtban et al., 2010). The carbohydrate fermentation test carried out shows positive results on several sugars tested, including glucose, sucrose, and lactose, indicating that these two bacterial isolates are able to ferment carbohydrates. Based on the indicators observed in this test, the media color changes from neutral green to yellow.

Conclusions

The phenotypic characters of the 12 mangrove sediment isolates all have a rod cell shape (bacillus), are Gram-positive, positive for glucose fermentation, and are motile. Eleven isolates are catalase positive, and there is only one isolate that is catalase negative, namely isolate JTMS.II2.

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