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BIOELECTRICITY GENERATION FROM BAMBOO HYDROLYSATE BY MICROBIAL FUEL CELLS USING *PSEUDOMONAS AERUGINOSA* PR3 FOR NATURAL RESOURCE CONSERVATION

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Abstract

Microbial fuel cell (MFC) is a green technology that allows recovery of electricity from wastes, thus, it can be applied in environmental conservation, natural resource preservation and bioenergy production. As an attempt to efficiently utilize the increasing amount of bamboo wastes as an input for energy production, this study investigated the electricity generation efficiency of a dual-chamber MFC in sugar conversion into electricity using Pseudomonas aeruginosa PR3 and hydrolysates from the acid pretreatment of Bambusa stenostachya Hack.. MFCs were inoculated with PR3, acclimated with glucose then operated with bamboo hydrolysates containing different total reducing sugars as the anolytes. Total sugar consumption of the MFCs for electricity conversion varied from 76.25 to 96.30% after 48 hours of operation in bamboo hydrolysates. Results from electrochemical analysis showed that MFC with 9.0 g/L sugars from bamboo hydrolysates had better electricity production, with the maximum open-circuit voltage of 620 mV, current density of 1092.08 mA/m^2 and power density of 91.16 mW/m^2. Microbial communities in these MFCs could effectively recover electricity from up to 9.0 g/L reducing sugars in bamboo hydrolysates, preliminarily illustrating the applicability of the MFC technology in natural resource conservation as a way to produce alternative energy sources from the treatment of bamboo-processing wastes.

Keywords: ***M****icrobial fuel cell; Pseudomonas aeruginosa; Bamboo hydrolysate; Reducing sugars*

Introduction

Bamboo is the common name of a family of large woody grasses closely associated with Southeast Asian cultures. Due to its adaptability, quick growth rate and short life cycle, bamboos have been involved in many industrial applications and suggested as a feedstock for profitable bioenergy production owing to their biochemical composition of energy crops along with the huge amount of bamboo wastes [1]. In fuel production, biomass should undergo the pretreatment process to break down the structure of lignocellulose and release fermentable sugars for efficient conversion into energy products. Among the pretreatment methods, hydrolysis using dilute acid can dispense sugars in a way which requires less budget, energy and fewer chemicals [1], hence its extensive studies in the conversion of bamboo residues into bioenergy for effective bamboo utilization, waste management and environment protection.

Microbial fuel cell (MFC) is a sustainable technology using microbes to generate electricity beside wastewater treatment and resource recovery. Various substrates can be used in MFCs, as long as they contain organic matters to support microbial metabolism [2]. Pure-grade

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substances are more readily consumable sources for the microbes; however, their high cost impedes their applicability in industrial-scale MFCs. On the other hand, wastewater or pretreated waste biomass is a more profitable and practical option thanks to its availability, low to almost no cost, substrate abundance and diversity. In addition, microbial decomposition of substrates or pollutants present in wastewater or hydrolysates from complex biomass can improve the effluent quality before discharge to the environment [2]. By directly turning wastes into electricity, MFC has unlimited potential in both environmental and natural resource conservation, in which less non-renewable fuels would be required to serve the current energy demands while the problem of biomass wastage and inefficient biomass waste handling would be addressed. Scaling-up and applications of MFCs have several challenges in the high costs and low power output [2], thus, more attentions are on the optimization of MFC components.

Pseudomonas aeruginosa is a ubiquitous Gram-negative and opportunistic pathogen [3], and PR3 is a *P. aeruginosa* strain reported to transform unsaturated fatty acids such as oleic acid, linoleic acid, etc., into dihydroxy fatty acids, which have many potential industrial applications [4]. *P. aeruginosa* is reviewed to be metabolically-versatile considering the plenty biochemical pathways which it uses to support aerobic growth and anaerobic survival; the latter is possible because of the strain's secretion of phenazine and its derivatives which aids in its energy conservation and maintenance of redox homeostasis [3]. These, along with the strain's strong formation of biofilms, make *P. aeruginosa* a potential exoelectrogen in MFCs [5]. When a mixed culture is used, *P. aeruginosa* biofilm provides a matrix of accumulated phenazines for other species to utilize as electron shuttles, enhancing the overall MFC current generations and power outputs [5]. A mixed culture can improve MFC performances as long as there is at least one exoelectrogen in the community [2, 5].

With these features, PR3 could be applied in MFCs to recover energy from sugar- and oil-rich wastewater, hydrolysates from pretreated lignocellulosic biomass or biomass residues after product-manufacturing at factories for clean energy production, environmental protection and resource preservation. Since not much research has been established on PR3 activities in MFCs, this study aimed to investigate the growth of PR3 and its electricity generation in MFC systems that used acid-pretreated bamboo biomass as the feedstocks. To evaluate MFC performances, electrochemical and biological analyses were included during acclimation in glucose and operation in bamboo hydrolysates.

Materials and Methods

Materials and culture conditions

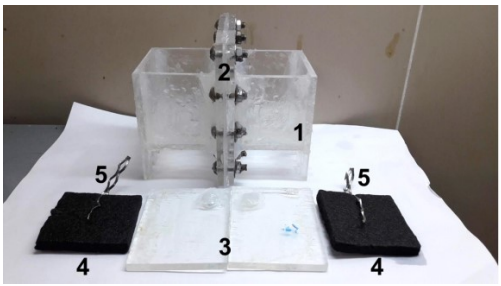
Pseudomonas aeruginosa strain PR3 (NRRL B18602) was taken from Chungbuk National University (Korea) and *Bambusa stenostachya* Hack. was collected from Phu An Bamboo Village (Binh Duong province, Vietnam). Media for PR3 growth and MFC anolytes were PBS 50mM, pH = 7 composed of 11.09g/L $Na_2HPO_4.12H_2O$, 2.58g/L KH_2PO_4, 0.14g/L KCl, 0.32g/L NH_4Cl, 0.21g/L $MgSO_4.7H_2O$, 0.08g/L $CaCl_2$, 10.0mL/L trace mineral mix of 0.286g/L H_3BO_3, 0.181g/L $MnCl_2.4H_2O$, 0.010g/L $ZnCl_2$, 0.008g/L $CuSO_4.5H_2O$ and 0.005g/L $Co(NO_3)_2.6H_2O$. Two carbon sources investigated in this study were analytical glucose and reducing sugars from bamboo hydrolysates, which were made by pretreating 1.0g of dried bamboo powder with 15.0mL of 3.5% (w/w) H_2SO_4 at 121°C for 1 hour using an autoclave [6]. The filtrate was diluted to desired total reducing sugar levels of 5.0, 7.0 and 9.0g/L in PBS 50mM pH = 7. The reducing sugars in bamboo hydrolysates included glucose, xylose, arabinose and various oligosaccharides from the breaking-down of cellulose and hemicellulose in bamboo powder during hydrothermal pretreatment in dilute sulfuric acid [6, 7].

Cryogenic PR3 was first revived in an overnight LB culture (10.0g/L tryptone, 5.0g/L yeast extract, 10.0g/L NaCl), then transferred to the buffered growth medium (PBS with 5.0g/L glucose, 1.0g/L yeast extract) in a 5% (v/v) inoculum size and incubated for 18 hours (28°C,

200rpm). After incubation, the working culture was centrifuged and resuspended in PBS to OD_{600} of 1.0 before transferring to the MFC anodes. Anaerobic growth was investigated with PR3 resuspended in PBS containing glucose (5G, 7G and 9G) and in bamboo hydrolysates diluted to 5.0, 7.0 and 9.0g/L of total reducing sugars (5B, 7B and 9B).

MFC design

Acrylic dual-chamber MFCs were constructed based on the previous design [8]. The chambers were separated by a Nafion-115 proton exchange membrane (65 × 45mm). Two graphite felt electrodes (50 × 50 × 5mm), which were connected to two titanium wires and into a circuit, were allowed a 10mm spacing between them with the membrane in the middle so as to minimize internal resistance (Fig. 1). Graphite felt electrodes and Nafion-115 membranes were pretreated [8] before MFC assembly.



(1) Two-chamber acrylic assembly
(2) Nafion-115 membrane
(3) Acrylic lids with stoppers
(4) Graphite felt electrodes
(5) Titanium current collectors

Fig. 1. Acrylic dual-chamber MFC assembly with its separate components

MFC performance

All MFCs in study were maintained at room temperature (30-33°C) and in an airtight condition. 120.0mL of resuspended PR3 culture was transferred to the anode chamber of each MFC with the conditions specified in Table 1 while a negative control (MB) was prepared using PBS and 5. g/L glucose only. MFCs underwent two acclimation stages, including the first acclimation constantly running on 5.0g/L glucose for 45 days (equivalent to 9 cycles) then the second acclimation stage with 5.0, 7.0 and 9.0g/L glucose for 16 days (equivalent to 6 cycles) (Table 1). The catholyte throughout performance was ferricyanide ($K_3[Fe(CN)_6]$) 50mM in PBS 50mM, pH = 7. MFCs were connected to a gradually increasing external resistance (100, 200, 500 and 1000Ω) during the first acclimation stage and a constant value (1000Ω) in the second acclimation stage. Voltage was monitored using a data acquisition system (Keithley Instruments, 2701); when MFC voltage decreased, the electrolytes were replaced and considered to have completed one cycle. Once MFCs stably achieved the maximum voltages for at least two consecutive cycles in the second acclimation, MFCs were allowed to operate without resistors for evaluation of their electricity generation. The conditions in the operation stage using bamboo hydrolysates as substitutes for glucose were listed in Table 1.

Table 1. Acclimation and operation conditions of MFCs

Substrate	**Glucose**						**Reducing sugars (bamboo hydrolysates)**		
Stage	**1st Acclimation**			**2nd Acclimation**			**Operation**		
	MFC	**PR3**	**(g/L)**	**MFC**	**PR3**	**(g/L)**	**MFC**	**PR3**	**(g/L)**
	MB		5.0	MB		5.0	MB		5.0
	M5G	×	5.0	M5G	×	5.0	M5GB		5.0
				M7G	×	7.0	M7GB		7.0
				M9G	×	9.0	M9GB		9.0

Note: × implies that PR3 was added to the anolyte of designated MFC.

Evaluation methods

Cell growth was measured via OD_{600} (PG Instruments, T60) and viable counts by plating on plate-count agar. Brief assessment of the bacterial community was done by Gram-staining.

Total reducing sugars were determined using Miller's colorimetric DNS assay [9] with the absorbance measured at 530nm. Sugar consumption rate was calculated according to equation (1) as follows:

$$\text{Percentage of sugar consumption} = [(C_0 - C_t) / C_0] \times 100\% \quad (1)$$

where: C_0 and C_t were defined as sugar concentrations (g/L) at the beginning and t hours from the beginning, respectively.

Electrochemical analyses included linear sweep voltammetry (LSV) and electrochemical impedance spectroscopy (EIS) performed using the multichannel potentiostats (Bio-Logic, VSP and VMP3B-5). LSV was done with 1mV/s scan rate from the maximum open-circuit voltage (OCV) to 0V [8], yielding a polarization curve that featured power density (P, mW/m^2) and current density (j, mA/m^2) which were calculated based on equations (2) and (3) as follows:

$$P = (V \times I) / A \quad (2)$$

$$j = I / A \quad (3)$$

where V (V) was the OCV, I (mA) was the corresponding current and A (m^2) was the electrode surface area (25cm^2). EIS were carried out shortly after medium replacement with the frequency range 100kHz-0.1Hz and a 10mV amplitude [8], resulting in a Nyquist plot on the EC-Lab software characterizing the internal resistance.

The biofilm formed on anodes was observed using scanning electron microscopy (SEM). Each anode following chamber disassembly was fixed with 2% (v/v) glutaraldehyde for 3 hours then washed thrice in PBS 50mM, pH = 7. The anodes after fixation was dehydrated in a step-wise manner with ethanol gradients of 30, 60, 90 and 99.5% (v/v) then dried at 80°C [8] and sputter-coated with gold prior to SEM analysis.

Results and Discussion

P. aeruginosa PR3 anaerobic growth

PR3 anaerobic growth in the MFC anolytes is illustrated in Figure 2.

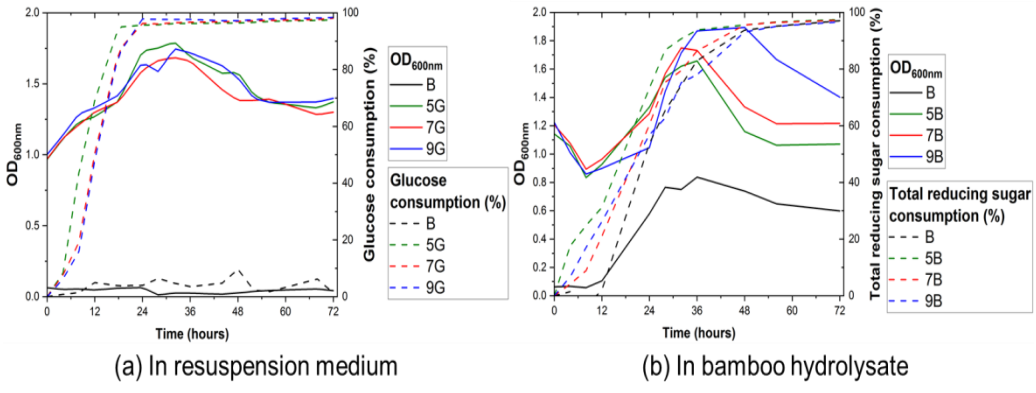


Fig. 2. Growth curve and sugar consumption during PR3 anaerobic growth

Cell density reached the maximum OD_{600} after 24 to 36 hours and over 95% of the initial glucose was consumed within this period. PBS containing 5.0g/L glucose (5G) gave the highest PR3 cell growth. On the other hand, PR3 cultures in bamboo hydrolysates took 48 hours to consume over 95% of the initial sugars, and the highest viable growth was provided by up to 9.0g/L total sugars (9B). This might have been due to the complex composition of bamboo hydrolysates [6, 7] causing an inhibitory effect on microbial growth, but the higher amount of reducing sugars in bamboo hydrolysates with higher concentrations could better support PR3 metabolism.

Performance evaluations

Influence of external resistance on MFC voltage

Figure 3 demonstrates how the voltage changed in the 1st Acclimation in response to the increase in external resistance. Voltages of PR3-inoculated MFCs were significantly higher than that of MFC without PR3 (MB), whose voltage was close to zero for 12 days. The increase in external resistance gradually enhanced voltage and quickened the start-up time, as the initially low external resistance favors electrogenic metabolism, anode colonization and biofilm formation while the increasing external resistance slowly increases the output voltage, biofilm thickness, power stability and decreases internal resistance [10, 11].

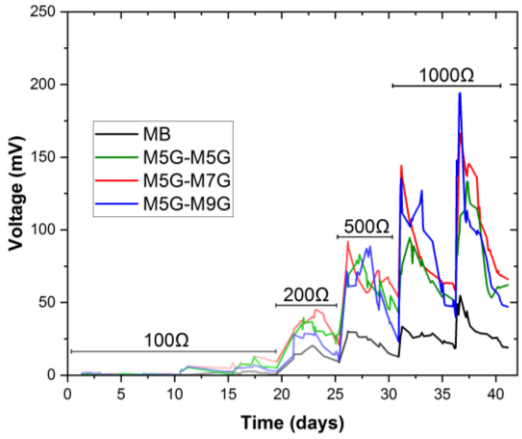


Fig. 3. MFC voltage in the 1st Acclimation according to external resistance

MFC voltage, sugar consumption and cell growth

In the 2nd Acclimation (Fig. 4), PR3-added MFCs had short start-up time and stable electricity production. The blank's voltage, however, took 23 hours to peak then became unstable, so PR3 addition helped accelerate the start-up time and stabilize electricity production. M5G and M7G had the highest OCVs and shortest adjustment durations while 9.0g/L glucose might be too overwhelming for PR3, which explained for M9G's lower OCV slightly prolonged adjustment time. The accelerated voltage was consistent with the rapid glucose consumption as over 95% of the initial glucose was consumed within 10 hours of performance, except for MB requiring 24 hours to degrade the same amount. Electricity production of MB was due to glucose self-oxidation and the slow increase in anolyte cell density (Appendix).

Figure 4b reveals that bamboo hydrolysates boosted MFC voltages but somehow slowed down adjustment time compared to acclimation in glucose, which might have been due to no PR3 addition in the Operation stage and nutrient overloads caused by the complexity of bamboo hydrolysates [6]. The slow voltage increase correlated with the slow sugar consumption because by the time the voltage peaked, only 29-36% of the initial sugars was used, so it was possible that the MFCs might have degraded the simple sugars first before using more complex ones in

bamboo hydrolysates later on [6]. The increase in MB's cell density hinted that there were bacterial cells from the unsterilized bamboo hydrolysates before MFC feeding, which benefited electricity production of all MFCs in study (Appendix).

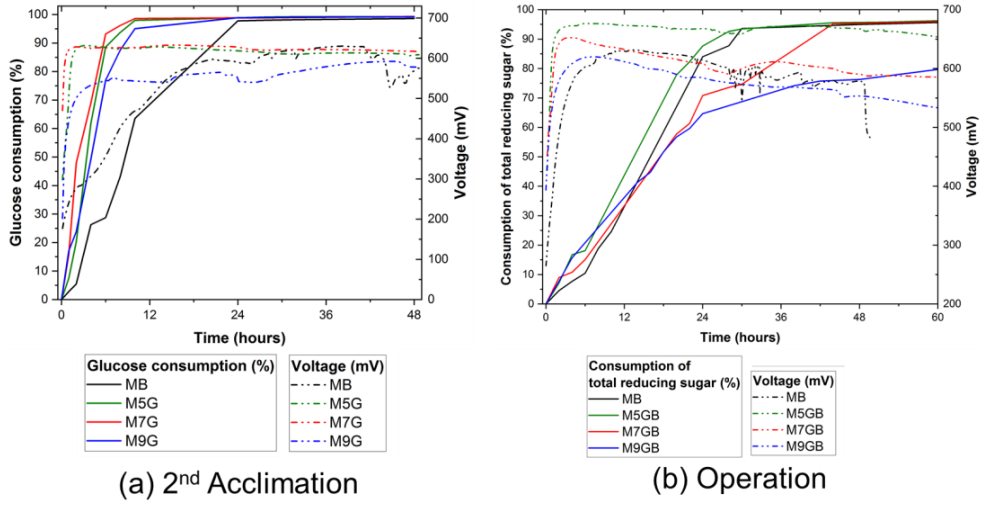


Fig. 4. Voltage and reducing sugar consumption in the last cycles of each performance stage

Polarization curves

Figure 5 and Table 2 are the results obtained from LSV scanning. Despite sharing the same amounts of sugars, OCV, P and j of MBs were lower compared to those of their respective M5G and M5GB, emphasizing the importance of routine PR3 transfers. The maximum OCV of M5GB from LSV was 593mV, which was lower than recorded during monitoring (677mV) as a result of improper attachment of the potentiostat's cables to M5GB's electrodes during performance of LSV analysis.

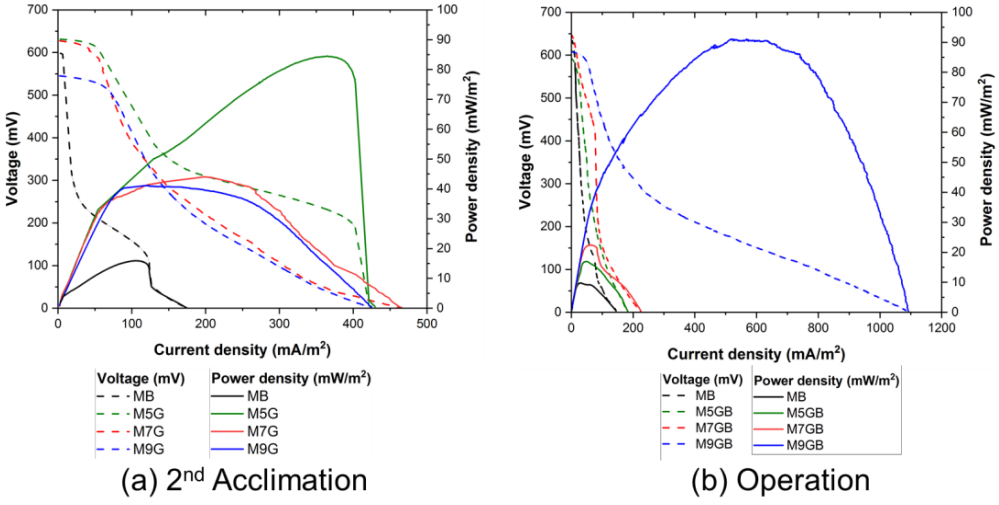


Fig. 5. Polarization curves of MFCs in each performance stage

Table 2. Electrical behaviors of MFCs resulting from LSV scans

Stage	MFC	OCV (mV)	P (mW/m^2)	j (mA/m^2)
2nd Acclimation	MB	598	15.90	175.90
	M5G	631	84.52	433.17
	M7G	627	44.12	467.54
	M9G	545	41.91	426.36
Operation	MB	633	10.04	144.89
	M5GB	593	17.82	183.25
	M7GB	649	33.57	225.71
	M9GB	609	91.16	1092.08

In the 2nd Acclimation, the maximum P of M5G was nearly two-fold higher than M7G and M9G while their maximum j values were quite similar; all of which were higher than MB (Fig. 5a), so M5G displayed the best electrochemical performance and the slightly reduced values of M9G were possibly due to the influence of nutrient overload. During the Operation stage, the increase in sugars from bamboo hydrolysates up to 9.0g/L brought along the increase in MFC electrical capacity, particularly, M9GB had the highest maximum P (91.16mW/m^2) and j (1092.08mA/m^2) out of all variables (Fig. 5b and Table 2), which could be attributed to its higher amount of monomeric carbohydrates compared to more diluted hydrolysates.

Generally, MFCs with 5.0g/L glucose and up to 9.0g/L total reducing sugars could give better power in acclimation and operation stages, respectively. When glucose was replaced with reducing sugars from bamboo hydrolysates, electricity production of MFCs improved significantly. Apparently, the bacteria in MFC anodes could adapt to the bamboo hydrolysate environment (Fig. 2b) and degrade the organic matters to generate electrons. The higher total reducing sugars in bamboo hydrolysates as determined by the DNS method could have been more beneficial to MFC performance. Considering the substrate costs and availability, bamboo hydrolysates would be more economical and practical than analytical glucose to apply in MFCs for the purposes of green energy recovery coupling with natural resource preservation and environmental protection, especially when bamboo post-processing wastes are used instead of the biomass from original bamboo trees.

Internal resistance

All MFCs displayed a similar Nyquist plot after EIS analysis, which included one semicircle (Fig. 6). The internal resistances of MB, M5GB, M7GB and M9GB were 86.53, 27.68, 50.24 and 26.04Ω, respectively. The establishment of an effective biofilm is known to reduce internal resistance [12], and since MB did not have PR3 addition, substrate degradation at the anode surface would be slower, leading to higher internal resistance and lower power outputs. M9GB had the smallest internal resistance, corresponding to its highest electrical capacity (Table 2). M5GB and M9GB had similar internal resistances but M9GB performance was more outstanding, which could be due to the interference M5GB suffered during LSV as explained previously or the fact that 5.0g/L total sugars in bamboo hydrolysates might have not been enough for effective operation. Although M7GB had its internal resistance higher than that of M5GB, its maximum power density was better, which could have been associated with its higher cell viability and substrate amounts.

Anodic bacterial community

Gram-staining of MFC samples including the anolytes, the biofilm swabs on anodes and the floating pellicles proved that the anodic bacterial community comprised of many types of cells, with the most predominant one being round, large and Gram-positive (Appendix). The presence of a microbial community while culturing PR3 in MFC enhanced electricity production as other species assisted *P. aeruginosa* PR3 in degrading complex substrates in bamboo hydrolysate while the strain facilitated extracellular electron transfer [2, 5].

SEM analyses are shown in Figures 7 and 8. M5GB, M7GB and M9GB showed uniform biofilms with dense microbial populations being linked by vicious substances while SEM

images of MB anode revealed only graphite fibers with some objects and no bacterial cells. With this observation, along with the increasing cell count in MB's anolyte over time, it was possible that these non-exoelectrogenic cells might have been unable to bind to the anode surface, hence no biofilm formation and low power output.

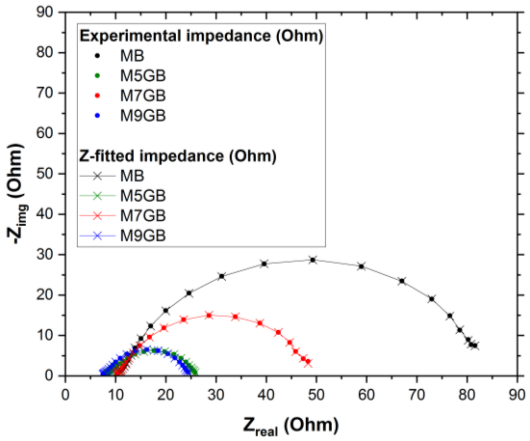


Fig. 6. Nyquist plot of MFCs in Operation

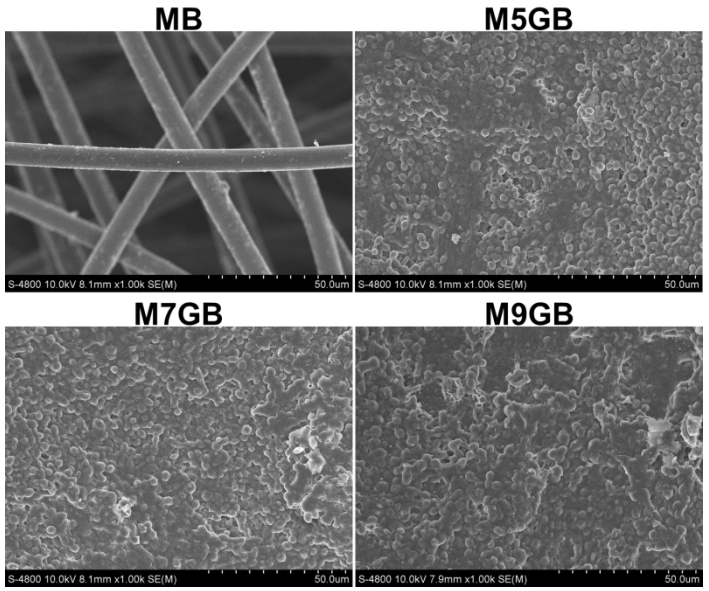


Fig. 7. SEM images of MFC anodes at 1000X magnification factor

The majority of cells captured by SEM in other MFCs were large and round, which was different from the common morphology of *P. aeruginosa* as observed by SEM in other studies [5] but agreed with our Gram-staining results. The overall biofilm structures of M5GB and M9GB had more void spaces compared to the compact structure of M7GB anodic biofilm. According to literature review, the presence of void spaces in biofilms could provide substrate, buffer and product exchanges, thus increasing biofilm viability in the innermost layers [11],

which could explain for the low internal resistance and high electrical features of M5GB and M9GB.

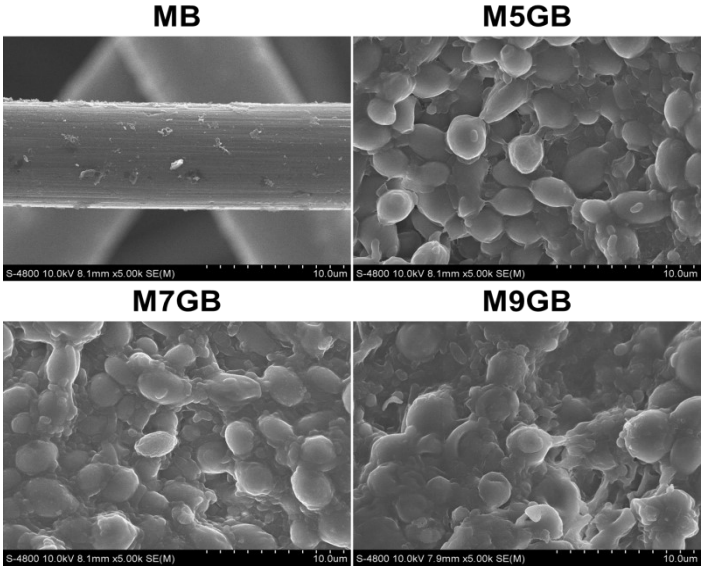


Fig. 8. SEM images of MFC anodes at 5000X magnification factor

The highly compact structure of M7GB biofilm might have caused its high internal resistance but the amount of substrates and cell density might have compensated and led to its average electricity production.

Conclusions

This current study has preliminarily investigated the bioelectricity generation from sugar degradation of *P. aeruginosa* PR3-based MFCs using hydrolysates from bamboo pretreatment. In general, reducing sugars obtained from bamboo hydrolysates are more advantageous as MFC feedstocks than glucose in terms of substrate costs, practicality and real-life applications, particularly in bamboo waste management, environmental conservation, resource preservation and smart resource utilization. Throughout performance, MFCs with PR3 additions have higher power outputs, quicker start-up time and longer operational stability. During acclimation, 5.0g/L glucose gives the best MFC performance (84.32mW/m^2, 433.17mA/m^2) while that during operation is provided by up to 9.0g/L total sugars in bamboo hydrolysate (91.16mW/m^2, 1092.08mA/m^2). The higher sugar contents in bamboo hydrolysates offer more superior substrate abundances to power MFCs compared to glucose, thus, if bamboo wastes were utilized in replacement of the original biomass, this MFC configuration would allow more bamboo wastes to be handled, leading to a reduction in pollution caused by biomass waste while electricity is produced in a sustainable manner. The microbial communities have not only PR3 but also other microbes present in the anode chamber, which has a positive influence on total sugar degradation and bioelectricity generation. Overall, the research has demonstrated the potential of bamboo hydrolysate as an input substrate for MFCs. Successful application of MFC technology would allow bamboo biomass utilization at full capacity, efficient bamboo waste management, environment protection and sustainable green energy production based on the carbohydrate fraction of bamboo-processing effluents. Further investigations stemming from this research would include evaluating MFC performance with higher sugar concentrations and optimizing operating conditions of PR3-based MFC feeding on bamboo hydrolysates.

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Appendix

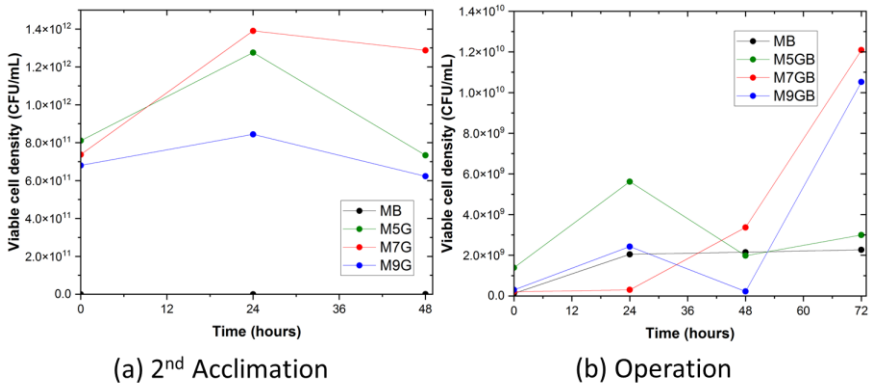

Fig. A1. Anolyte cell growth during MFC performance

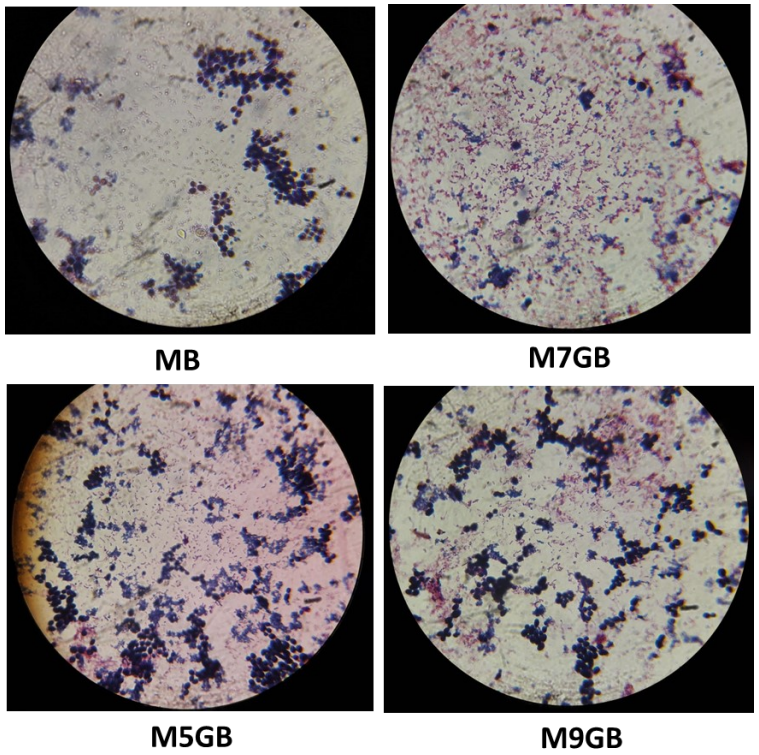

Fig. A2. Gram staining results of MFC anolytes following the final operation cycle in bamboo hydrolysate

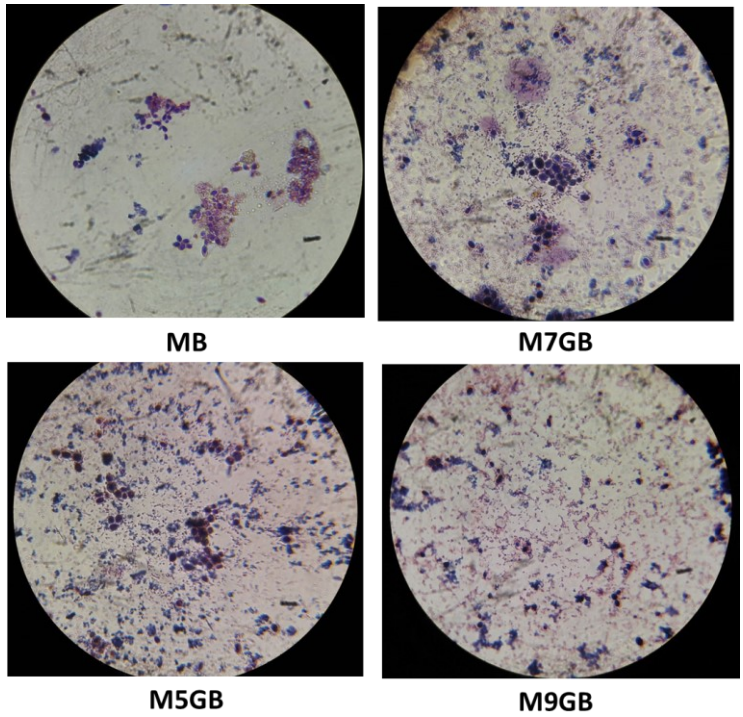

Fig. A3. Gram staining results of anodic biofilm swabs following the final operation cycle in bamboo hydrolysate and MFC disassembly

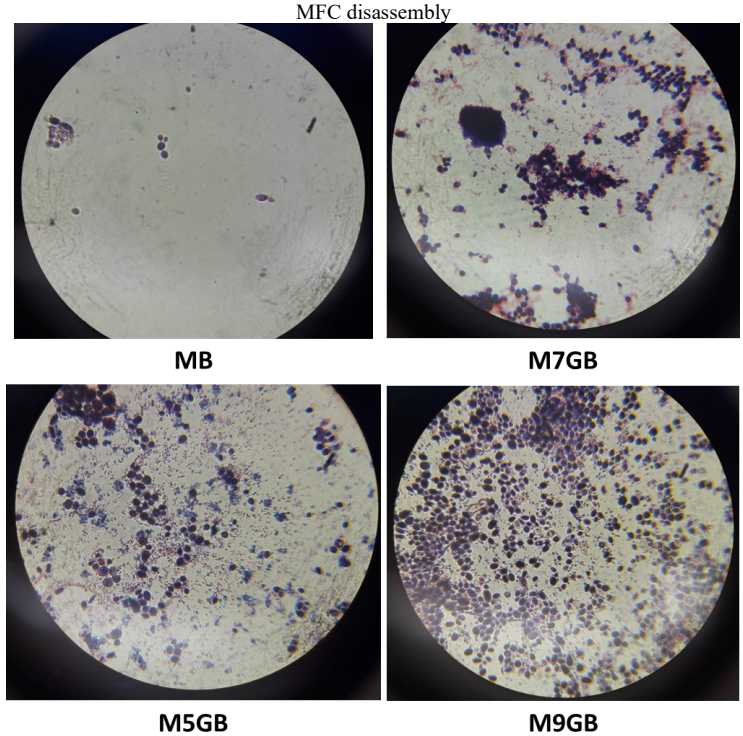

Fig. A4. Gram staining results of anodic floating pellicles following the final operation cycle in bamboo hydrolysate and MFC disassembly