BIODIVERSITAS
Volume 21, Number 4, April 2020
Pages: 1686-1696

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d210450

Isolation and selection of cellulolytic bacteria from rice straw for consortium of microbial fuel cell

NUR SYAFIRA KHOIRUNNISA♥, SYAIFUL ANWAR[1], DWI ANDREAS SANTOSA
Department of Soil Science and Land Resource, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8629354, Fax.: +62-251-8629352, ♥email: n.syafira.kh@gmail.com

Manuscript received: 2 January 2020. Revision accepted: 27 March 2020.

Abstract. *Khoirunnisa NS, Anwar S, Santosa DA. 2020. Isolation and selection of cellulolytic bacteria from rice straw for consortium of microbial fuel cell. Biodiversitas 21: 1686-1696.* Cellulose such as in rice straw can be utilized as an organic substrate in Microbial Fuel Cell (MFC) to generate electricity by microorganisms as a biocatalyst. This research aimed to get cellulose-degrading bacteria with high capability to degrade rice straw and able to be used as consortium with exoelectrogen bacteria in Microbial Fuel Cell. The stages of research included: (i) isolation of the bacteria using carboxymethylcellulose (CMC) agar medium, (ii) selection of the isolates for that purpose, (iii) enzyme assay and MFC performance test, and (iv) identification of selected isolate. There were 125 isolates that were obtained. Selection based on the ability to degrade cellulose as indicated by clear zone on CMC medium resulted in 23 isolates. Ten isolates belong to anaerobic facultative bacteria were selected. Three of them were synergistic with exoelectrogen bacteria. The three isolates were tested for exoglucanase (Avicel) and total enzyme activity (Filter Paper) with the highest results were 6.21 U/mL (isolate J404) and 5.88 U/mL (isolate J401), respectively. The optimum MFC performance was achieved by one isolate, J401, which produced highest voltage of 40.8 mV and a power density of 0.33 mW/m^2. The best isolate, J401, was identified as *Xanthomonas translucens* based on 16S rRNA method.

Keywords: Bacteria, cellulose, decomposition, degrading, exoelectrogen

INTRODUCTION

The biomass residue of agriculture production contains large amounts of biochemical energy in the form of cellulose and lignin. The last data from FAO shows that rice production in the world in 2017 reaches 3832 million tons (FAO 2018). The increase in agricultural production correlates with the increase of organic waste, such as rice straw. The percentage of rice from the total biomass of rice plants is 65-72 % (Singh et al. 1995). There are around 1149.6 million tons of rice straw in the world.

The degradation of lignocellulosic biomass such as rice straw is done by the collaboration of many microorganisms, including diverse fungal and bacterial genera producing a variety of cellulolytic enzymes under aerobic or anaerobic conditions. Cellulolytic microorganisms can degrade cellulose-based plant litter (Soares et al. 2013). Cellulose is a linear polymer of glucose linked through ß-1,4-glycosidic linkages that is the primary structural material of the plant cell wall and is the most abundant carbohydrate in nature (Saha et al. 2006). Cellulose is degraded by a biological process controlled and processed by the enzymes of the cellulase group (Gupta et al. 2012). Main member of this group includes endoglucanase, exoglucanase or cellobiohydrolase, and ß-glucosidase (Bayer et al. 2007). The endoglucanase is responsible for cleavage of β-1,4-glycosidic bonds along a cellulose chain. The exoglucanase is required for cleavage of the non-reducing end of a cellulose chain and splitting of the elementary fibrils from the crystalline cellulose. The β-glucosidase hydrolyzes cellobiose and water-soluble cellodextrin to glucose (Bhat and Bhat 1997). There have been many studies that isolate cellulose-degrading bacteria including isolation from plant litter soil (Waghmare et al. 2018), sediment of mangrove swamps (Chantarasiri 2015), and the gut of termites (Upadhyaya et al. 2012).

One kind of alternatives to utilize biomass waste, especially cellulose, is Microbial Fuel Cell. Microbial Fuel Cell (MFC) is a bioelectrochemical reactor that converts directly organic material into electricity. The MFC can utilize some types of waste especially agro wastes to generate electricity. This system uses active organisms as biocatalysts that have the capability of generating electricity (Logan and Regan 2006). The MFC commonly used organic materials including carbohydrate groups such as glucose, sucrose, and starch; low molecular weight organic acids such as acetate, oxalate, and fumarate; and amino acids. Several experiments have tried to use cellulose in various forms as organic material in the MFC such as the ones done by Kawale et al. (2017) who used carboxymethylcellulose (CMC), Huang and Logan (2008) who used paper recycling wastewater, or Thygesen et al. (2011) who used wheat straw.

This research aimed to get facultative cellulose degrading bacteria with high capability to degrade rice straw and able to be used as consortium with exoelectrogen bacteria in Microbial Fuel Cell. The rice straw is used as substrate in the MFC therefore we choose isolation sources for cellulolytic bacteria from rice straw with different degradation levels.

MATERIALS AND METHODS

Sample collection and isolation of isolates

Sample collection

Decomposed rice straw samples were collected from two fields. There were four samples; one with low decomposition level, one with medium decomposition level, and two with high decomposition level. The rice plant was planted in paddy soil, in Bogor, Indonesia. Sample with high decomposition level was expected to have more potential cellulolytic isolates. The decomposition level was determined by physicochemical properties of rice straw. The parameters were the easiness of rice straw to break down upon squeezing and C/N ratio. C/N ratio was calculated by divided organic carbon (OC) with total nitrogen (TN). Organic carbon was determined by Walkey and Black method (Walkley and Black 1934; FAO 2019) and total nitrogen was determined by Kjeldahl method (Kjeldahl 1883; Sáez-Plaza et al. 2013).

The rice straw easily squeezed to pieces was considered as straw with high decomposition level, while the one difficult to squeeze was considered as straw with low decomposition level. The rice straw with low decomposition level was characterized by intact rice straw. Whereas the rice straw in high decomposition level could not be distinguished from initial morphological shape of the rice plant. Besides, the color of the physiological solution mixed with samples of rice straw with low decomposition level is clearer than straw with high decomposition. The rice straw with low decomposition level has higher C/N ratio than rice straw with high decomposition level. All samples were collected in a sterile container and transported under aseptic conditions to the laboratory for further investigations.

Isolation of cellulose-degrading bacteria

Cellulose degrading bacteria were isolated using serial dilution and spread plate technique (Rawway et al. 2018). Ten grams of samples were put into an Erlenmeyer containing 90 mL sterile physiological solutions (0.85% w/v NaCl). The solutions were homogenized using a shaker for 30 min. The sample suspension was taken as much as 1 mL and was put into a test tube containing 9 mL of physiological solution and homogenized by a vortex. Serial dilution was made up until the 10^6 series. One hundred microliter sample suspension was inoculated on solid media (in 1 liter of media containing: 1 g KH_2PO_4, 0.5 g K_2SO_4, 0,5 g NaCl, 0.01 g $FeSO_4$, 0.01 g $MnSO_4$, 1 g $(NH_4)NO_3$, 10 g CMC, dan 20 g agar) by spread plate method. Petri dishes were incubated at ambient temperature for 3 until 5 d. When several colonies grew, the agar medium was stained with 1% (w/v) Congo red for 15 min and was washed with 1 M NaCl for 15 min (Teather and Wood 1982). Colonies that have cellulolytic ability were colonies that formed clear zones after the staining process. The isolates that have cellulolytic ability were purified by the quadrant-streak plate method to obtain a single colony. Then, the isolates were selected based on colony morphology (color, form, elevation, and margin). Isolates that have different morphologies were stored.

Selection stages

Cellulolytic activity assay

This test aimed to know the capability of the isolate to degrade cellulose by observing the clear zone in CMC agar. The cellulolytic activity was tested by growing the collected isolates on the CMC agar medium by a point method that repeated four times. Inoculants were incubated for 5 d at ambient temperature. After that, the plate was stained by 1% Congo red and was washed with 1 M NaCl for 15 min respectively (Teather and Wood 1982). Isolates that have cellulolytic activity showed a clear zone on the media. The ability to degrade cellulose was calculated based on the ratio of the clear zone diameter to colony diameter (Dantur et al. 2015).

Oxygen tolerance determination

This test aimed to recognize the oxygen tolerance of each isolate and to obtain facultative anaerobic bacteria using liquid medium (Parker et al. 2017). The facultative anaerobic bacteria have a wider oxygen tolerance range than aerobic and anaerobic bacteria. Then, the exoelectrogen bacteria that were used in this study, *Staphylococcus saprophyticus* ICBB 9554, were also facultative anaerobic bacteria. Ten mL of Nutrient Broth (NB) liquid media is prepared in a test tube. The media was sterilized using an autoclave at temperature of 121 ^oC, the pressure of 1 atm for 20 min. Then as much as one loop of the pure isolate was inoculated into NB media. The media was incubated at ambient temperature for 48 h. The position of bacterial growth was monitored to determine the type of oxygen tolerance.

Synergistic test

This test aimed to discover cellulolytic bacteria that were synergy with exoelectrogen bacteria so these two types of bacteria can be used as a consortium in MFC system. The exoelectrogen bacteria used in this study were *Staphylococcus saprophyticus* ICBB 9554 which was isolated from the rice soil ecosystem in Banten, Indonesia and have studied for MFC system before. The synergistic test was conducted by the disc diffusion method in two ways (Venkadesan and Sumathi 2015). The first synergistic test was between cellulolytic bacteria with *Staphylococcus saprophyticus* ICBB 9554. *Staphylococcus saprophyticus* ICBB 9554 was targeting bacteria and the testing bacteria was cellulolytic bacteria. Whereas the second test was the opposite of the first test. The bacteria target was cellulolytic bacteria and the testing bacteria was *Staphylococcus saprophyticus* ICBB 9554.

Some isolates of cellulolytic bacteria from the previous selection stage and *Staphylococcus saprophyticus* ICBB 9554 bacteria were inoculated in 25 ml NB media by taking one loop from stock culture. The isolates were incubated overnight (ON) and homogenized in a shaker. The culture media for the paper disc diffusion method was Nutrient Agar (NA). The petri disc containing solid NA was divided into 6 parts for the first test and into 3 parts for the second test. On the NA media, the bacteria target was spread by taking 0.1 ml of bacterial supernatant from NB media. The 6 mm diameter of paper disc was submerged in the

supernatant of the testing bacteria. The testing bacteria was placed over the targeting bacterial scratch. The bacteria strain with antagonist traits showed a clear zone surrounding the other strain and the synergism bacteria showed no inhibitory zone.

Test for potential isolate in MFC

Enzyme assay

The enzyme assay was conducted to measure the level of enzyme activity of the isolates. The test included exoglucanase activity and total enzyme activity. Initially, a crude enzyme had to be prepared. The crude extract of the enzyme was obtained by growing the isolates in the Mandels media (Mandels and Reese 1957) (in 1 liter of media containing: 14 g $(NH_4)_2SO_4$, 20 g KH_2PO_4, 3 g $MgSO_4.7H_2O$, 3 g urea, 30 g $CaCl_2$, 0.5 g $FeSO_4$, 1.6 g $MnSO_4$, 1.4 g $ZnSO_4$, 2 g $CoCl_2$, dan 5 g rice straw powder as a carbon source). It was incubated at ambient temperature for 5 d and was shaken in a 150 rpm shaker. The crude cellulase enzymes were harvested by centrifuging the media at a speed of 3634 x g at ambient temperature for 30 min then supernatants were taken.

The quantitative exoglucanase enzyme and total enzyme activity assay were carried out by measuring reducing sugars used 3,5-dinitrosalicylic acid (DNS) method (Miller 1959). Exoglucanase enzyme activity test used Avicel as a substrate (Dantur et al. 2015) and total enzyme activity used paper filter Whatman No 1. as a substrate (Ghose 1987). The exoglucanase enzyme activity test was carried out by mixing 1 mL enzyme filtrate, 1 mL phosphate buffer pH 7.00, and 1% microcrystalline substrate (Avicel) then incubated for 1 h at 60 ^oC. Then 3 mL of DNS (Dinitro Salicylic Acid) solution was added and was homogenized. The mixture solution was put into boiling water for 10 min. The total cellulase enzyme activity test was carried out similarly to the exoglucanase enzyme activity test. The Avicel substrate was switched with filter paper Whatman no. 1 and incubation were carried out for 30 min at 45 ^oC. The control was prepared by adding 1 mL of the filtrate enzyme after adding 3 mL of DNS. The blanco was prepared by mixing 2 mL distilled water, 1 mL buffer, and 3 mL DNS. All mixtures were put into boiling water for 10 min for color development. All of the mixtures were absorbed at a wavelength of 540 nm. Standard absorbance curves were performed using glucose solutions with concentrations of 0, 50, 100, 150, 200, 250, 300, and 350 ppm. One unit (U) of the enzyme activity was defined as the amount of enzyme releasing 1 μmol of reducing sugars equivalent to glucose per minute during the reaction.

Microbial fuel cell performance test

Microbial Fuel Cell performance test was conducted to recognize which one of the cellulolytic isolates has the highest performance by producing high electrical voltage. The selected isolates of cellulolytic bacteria and *Staphylococcus saprophyticus* ICBB 9554 were inoculated on 50 mL of NB media by taking 1 loop culture stock. The inoculants were incubated during one night (ON) at ambient temperature.

The MFC system in this study was constructed with a dual-chamber design based on the design of Razaei et al. (2009) with some modifications. The MFC chamber was assembled by acrylic material with a size of 6 cm x 6 cm x 7.5 cm and the capacity was 250 mL in each anode and cathode area. The anode and cathode compartments were separated by Nafon 117® Proton Exchange Membrane (PEM) (Lyntech, United States). The electrodes were carbon fiber at the anode and cathode with a cross-sectional area of 50 cm^2. The anode and the cathode were connected by an external circuit using copper wires to transfer an electron from the anode to the cathode. An additional 1,000 Ω external resistor was added. A schematic picture of MFC system was shown in Figure 1.

The PEM Nafion 117® membrane was treated by preliminary treatment refers to Mazzapioda et al. (2009). The first step was boiling the PEM membrane in 3% v/v hydrogen peroxide (H_2O_2) solution, then in 1 M H_2SO_4 solution, and finally in distilled water. Each treatment was done for 1 h. The membrane was stored in distilled water before being used. The membrane was stuck in the chamber by a seal. The chamber was sterilized by spraying alcohol followed by sterilization using a UV light in laminar airflow. The exposed process was carried out for 45 min. Carbon fiber electrodes were sterilized by spraying alcohol and burning on a bunsen burner.

The anode compartment contained 0.3% w/v rice straw (100 mesh size) dan 0.1 M KH_2PO_4. While the cathode compartment was filled by 0,1 M $KMnO_4$ dan 0.1 M KH_2PO_4. Both solutions were sterilized using an autoclave under 1 atm pressure, the temperature of 121 ^oC, for 20 min. Both solutions were poured aseptically on each side of the compartment. After that, 5 mL of cellulolytic isolate culture was added to the anolyte suspension. The MFC chamber was incubated for 72 h and after that 5 mL of *Staphylococcus saprophyticus* ICBB 9554 was added.

The MFC performance was monitored by measure electric current parameters (I) and voltage (V) using a multimeter every 8 h during the incubation period. The magnitude between current and voltage was validated by the formula I = V / R, where R was the resistance of an external resistor whose value was equal to 1,000 Ω. Then the power (P) was calculated using the formula P = IV. Power density is normalized by dividing the power value by the area of the anode (Razaei et al. 2009).

Identification using sequence of 16S rRNA

DNA isolation from bacterial colonies and PCR amplification was completed simultaneously using a direct PCR Kit (KOD FX Neo, Toyobo) following the Kit protocol. The PCR machine used was Eppendorf's master cycler and the primer used was a universal primary 16F27 (5'-AGAGTTTGATCMTGCCTCAG-3'). Each reaction mixture (50 µL) contained 25 µL PCR buffer, 10 µL dNTP (2 mM), 1.5 µL primers, 1 µL KOD FX Neo (1 U/mL), 2 µL isolated DNA, and PCR grade water until final volume reached 50 µL.

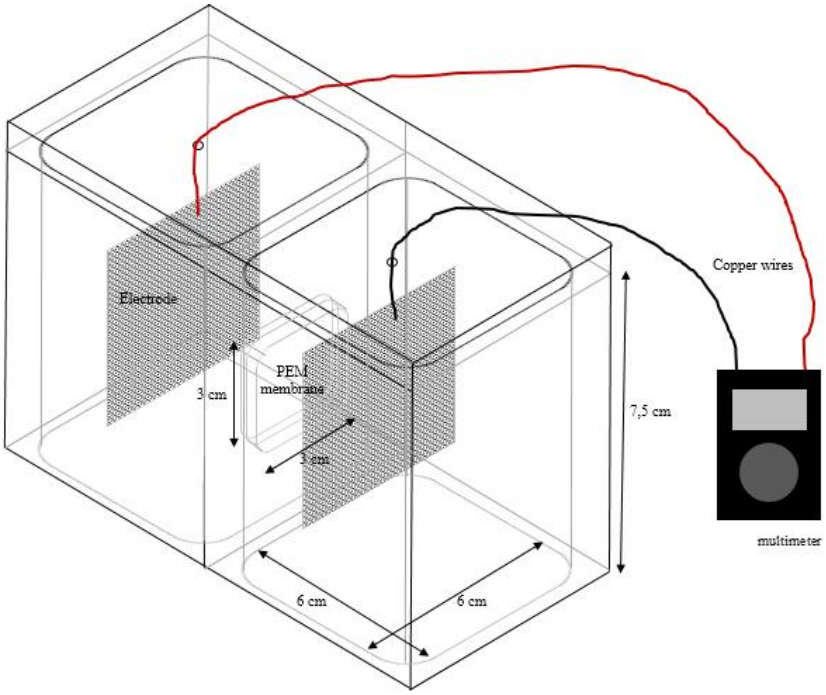

Figure 1. Schematic picture of MFC system

The cycling conditions were set up as follows: pre-denaturation of 94 ºC for 2 min, followed by 35 cycles of 98 ºC for 10 s, annealing at 53 ºC for 30 s, elongation at 68 ºC for 30 s, and final elongation at 72 ºC for 10 min. After amplification, 3 µL PCR product was run in 1% agarose gel and visualized using electrophoresis and captured using gel doc.

PCR product purification process, sequencing cycle up to the reading of the sequence of nitrogen bases was carried out by a sequencing service company (PT. Genetika Science). The data of 16S rRNA gene sequences were compared with the database at GenBank using the BLAST-N search program in National Center Biotechnology Information (http: //www.ncbi.nlm.nih.gov). The 16S rRNA gene sequences were aligned and the phylogenetic tree was constructed using MEGA6 software.

RESULTS AND DISCUSSION

Sample collection and isolation of isolates

The rice straw decomposition characterization by C/N ratio is shown in Table 1. The rice straw with low decomposition level has C/N ratio higer than rice straw with low decomposition level. The C/N ratio is one of the parameters that is used to assess the rate of decomposition and the decrease of C/N ratio due to mineralization of organic matter (Jusoh et al. 2013). Then total of 125 cellulolytic bacteria grew on the CMC agar medium was obtained. Total isolates from the rice straw with a higher decomposition level were more than that from the rice straw with a lower decomposition level. But after initial selection based on morphological likeness, the amount resulted from 55 isolates (Table 1). Those isolates were purified by the quadrant-streak plate method.

Selection stages

Cellulolytic activity assay

The result of cellulolytic activity showed that only 23 isolates which showed the formation of a clear zone from 55 collected isolates. It proved that not all collected isolates forming clear zones during isolation due to the isolates did not grow when purified. The clear zone ratio to diameter colony was shown in Figure 2.

Table 1. List of cellulose-degrading bacteria from rice straw with different decomposition level

Isolation sources	C/N ratio	Total isolate	Total morphological selection
Rice straw with low decomposition level	46,62 ± 1,99	19	9
Rice straw with medium decomposition level	32, 50 ± 2,42	32	16
Rice straw with high decomposition level	22,75 ± 0,08	41	17
Rice straw with high decomposition level*	25,12 ± 3,38	33	13
Total		125	55

Note: * the rice straw was collected from different location

The value of the clear zone ratio formed by 23 isolates ranged from 1.3 to 6.23. According to Figure 2, it showed that the isolates which have the highest clear zone ratio were J301, followed by J401, J104, J403, and J209 with the ratio values of 6.23, 5.24, 4.77, 4.45, and 3.61, respectively. Liang et al. (2014) reported the results of his research on cellulose-degrading bacteria with a wide inhibition zone ratio ranging from 6 to 17. However, the results from this research were relatively similar to research of Peristiwati (2018) with a range value of 1.07-1.32.

The clear zones formed from several isolates have different colors. There were orange clear zones that were brighter than CMC colored red by Congo red and there were white clear zones. Shil et al. (2014) reported the results of his research on the isolation of bacteria having cellulose degradation ability gave a white clear zone. Whereas Huang et al. (2012) reported that the clear zone formed in their research was orange.

Oxygen tolerance determination

In the test of oxygen tolerance, it can be seen (Figure 3) that the facultative anaerobic bacteria isolates present a more turbid color to the medium compared to the control. Then, compare to controls, aerobic or microaerophilic groups looked similar. The difference between aerobic and microaerophilic was a film or pellicle under the surface of the media. The recapitulation of oxygen tolerance and colony morphology was presented in Table 2. The facultative anaerobic bacteria were chosen for further experiments.

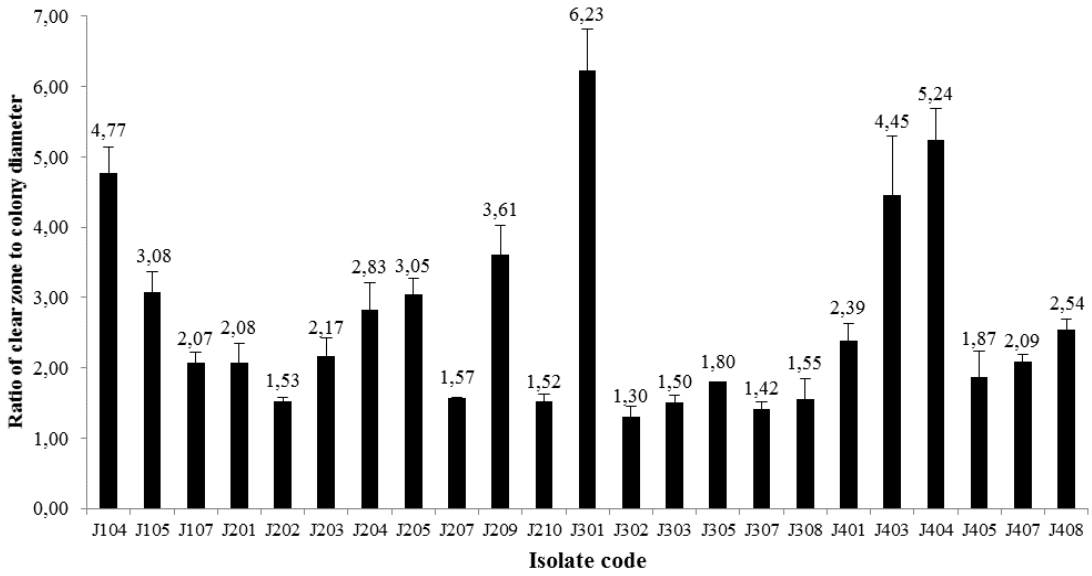

Figure 2. The ratio of a clear zone to diameter colony

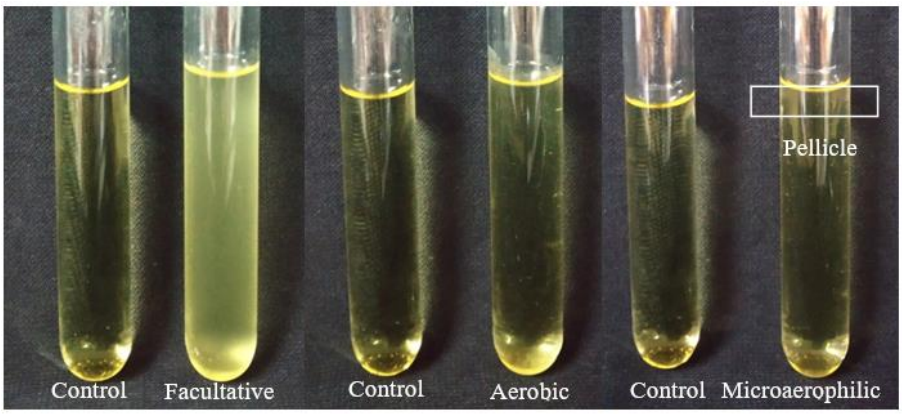

Figure 3. The appearance of oxygen tolerance from collected isolates compared by control. Facultative isolates showed turbid in a fluid medium and aerobic or microaerophilic isolates showed the same condition with control and have pellicle on the surface for microaerophilic

Table 2. Colony morphology and oxygen tolerance of the selected cellulose-degrading bacteria

No.	Isolate	Condition of NB	Oxygen tolerance	Color	Form	Elevation	Margin
1	**J104**	**Turbid**	**Facultative**	**White**	**Circular**	**Raised**	**Entire**
2	**J105**	**Turbid**	**Facultative**	**White**	**Circular**	**Raised**	**Entire**
3	**J107**	**Turbid**	**Facultative**	**White**	**Circular**	**Raised**	**Entire**
4	J201	Pellicle	Microaerophilic	Yellow	Irregular	Raised	Undulate
5	J202	Pellicle	Microaerophilic	Pink	Circular	Raised	Entire
6	J203	Pellicle	Microaerophilic	White	Circular	Raised	Entire
7	J204	Pellicle	Microaerophilic	White	Circular	Raised	Entire
8	**J205**	**Turbid**	**Facultative**	**White**	**Irregular**	**Flat**	**Curled**
9	**J207**	**Turbid**	**Facultative**	**Transparent**	**Irregular**	**Raised**	**Entire**
10	**J209**	**Turbid**	**Facultative**	**Yellow**	**Circular**	**Raised**	**Entire**
11	**J210**	**Turbid**	**Facultative**	**White**	**Circular**	**Raised**	**Entire**
12	J301	Pellicle	Microaerophilic	White	Circular	Raised	Entire
13	J302	Flock	Aerobic	Transparent	Irregular	Flat	Curled
14	J303	Flock	Aerobic	White	Circular	Raised	Entire
15	**J305**	**Turbid**	**Facultative**	**Yellow**	**Irregular**	**Flat**	**Curled**
16	**J307**	**Turbid**	**Facultative**	**Pink**	**Circular**	**Raised**	**Entire**
17	J308	Flock	Aerobic	Pink	Circular	Raised	Entire
18	**J401**	**Turbid**	**Facultative**	**Yellow**	**Circular**	**Raised**	**Entire**
19	J403	Flock	Aerobic	Yellow	Circular	Convex	Entire
20	J404	Pellicle	Microaerophilic	White	Circular	Convex	Entire
21	J405	Pellicle	Microaerophilic	White	Circular	Convex	Entire
22	J407	Flock	Microaerophilic	White	Irregular	Raised	Undulate
23	J408	Pellicle	Microaerophilic	Yellow	Irregular	Raised	Undulate

Synergistic test

The synergistic test results of cellulolytic bacteria against *Staphylococcus saprophyticus* ICBB 9554 bacteria can be seen in Figure 4. There were 7 isolates of cellulolytic bacteria that formed inhibitory zones against *Staphylococcus saprophyticus* ICBB 9554. The colonies with the highest ratio of inhibitory zones to diameter colony were J205, J107, and J209, with value of 3.68, 2.64, and 1.80, respectively. On the other hand, the test of *Staphylococcus saprophyticus* ICBB 9554 against 10 isolates of cellulolytic bacteria showed different results that were only 2 isolates forming inhibitory zones. The two isolates were J207 and J209 with inhibition zone diameter ratio to colony diameter of 1.05 and 1.07. The five isolates that formed inhibition zones previously namely J104, J107, J205, J210, and J305 were not indicated to form inhibitory zones in this second test. Based on that results, there were three isolates (J105, J401, and J404) that were synergistic with the exoelectrogen bacteria.

Test for potential isolate in MFC

Enzyme assay

The enzyme activity of 3 selected cellulolytic bacteria was measured. The selection was done based on high clear zone produced in Congo red test and synergistic test. Enzyme activity for Avicel substrate ranged from 5.16-6.21 U/mL while the activity of filter paper substrates ranged from 3.00-5.88 U/mL. The highest enzyme activity test on the Avicel substrate was obtained by isolates J404, followed by J401 and J105 with values of 6.21 U/mL, 6.03 U/mL, and 5.41 U/mL, respectively. The enzyme activity on filter paper substrate showed that the isolates of J401, J404, and J105 have the highest activity with values of 5.88 U/mL, 5.34 U/mL and 4.53 U/mL, respectively. This condition was better than research from Waghmare et al. (2018) wherein the filter paper test produced an enzyme activity of 0.15 U/mL and in Avicel produced activity of 2.52 U/mL and the research of Gupta et al. (2012) for filter paper substrates produced the highest activity value of 0.196 U/mL.

Microbial Fuel Cell Performance Test

The Microbial Fuel Cell Performance was showed by electrical voltage and power density profiles of the co-culture isolates of cellulose-degrading bacteria and exoelectrogen bacteria, *Staphylococcus saprophyticus* ICBB 9544. The value of the electrical voltage can be seen in Figure 6a. while the value of the power density can be seen in Figure 6b. The values shown on the graphic were the average values of both voltage and power density with three replications.

Figure 6 showed MFC performance started from 16 h. At that time the electrical current and voltage were stable. The performance of electrical voltage from each cellulolytic bacteria isolates was different. MFC performance from isolate J105 showed low voltage value at the initial incubation period until the 96 h then the voltage increased after 104 h with an average value of 8.13 mV and continued to increase until the highest value of 11.6 mV at 128 h. After that, it decreased dramatically to 1.6 mV. Different pattern was showed by strain J404. There was a

voltage enhancement at 24 h with 4.47 mV then increased to 25.80 mV and continued to increase with a maximum increase of 37.57 mV at 56 h. The voltage of isolate J401 was high at the beginning with a value of 40.80 mV then decrease at 32 h with 21.23 mV but was stable at a value of 13-15 mV from 64 h until the end of incubation period.

Furthermore, the highest power density value of the MFC system was achieved by isolate J401 at 24 h incubation period with a power density of 0.33 mW/m^2. Isolate J404 showed an attractive power density pattern that was low at the beginning, then increased some time with the highest value of 0.32 mW/m^2 at 56 h, and then decreased until its value was close to 0 mW/m^2. The result of this test was corresponding to the study of Khawdas et al. (2017) with cellulose as a substrate and *Cellulomonas fimii* as a biocatalyst which produced maximum power density of 0.74 ± 0.07 mW/m^2.

Identification using sequence of 16S rRNA

Isolate J401 was selected based on clear zone test, and was facultative anaerobic bacteria and synergistic with exoelectrogen bacteria. The J401 isolate has also a good performance in producing voltage and power density.

The identification of 16S rRNA was initiated by isolating the bacterial DNA of J401 and amplified using universal primers that produced amplicon of around 1500 bp. Furthermore, a partial-sequencing of 16S rRNA was done and compared with sequence data from the gene banks (NCBI).

The phylogenetic tree (Figure 7) was constructed using maximal likelihood with model of Kimura 2-parameter. The isolates J401 has homology identity of 100% and query recovery of 98% with *Xanthomonas translucens* (NR_036968.1). The isolate, J401, was deposited at the ICBB (Indonesian Center for Biodiversity and Biotechnology) with accesion number of ICBB 9762.

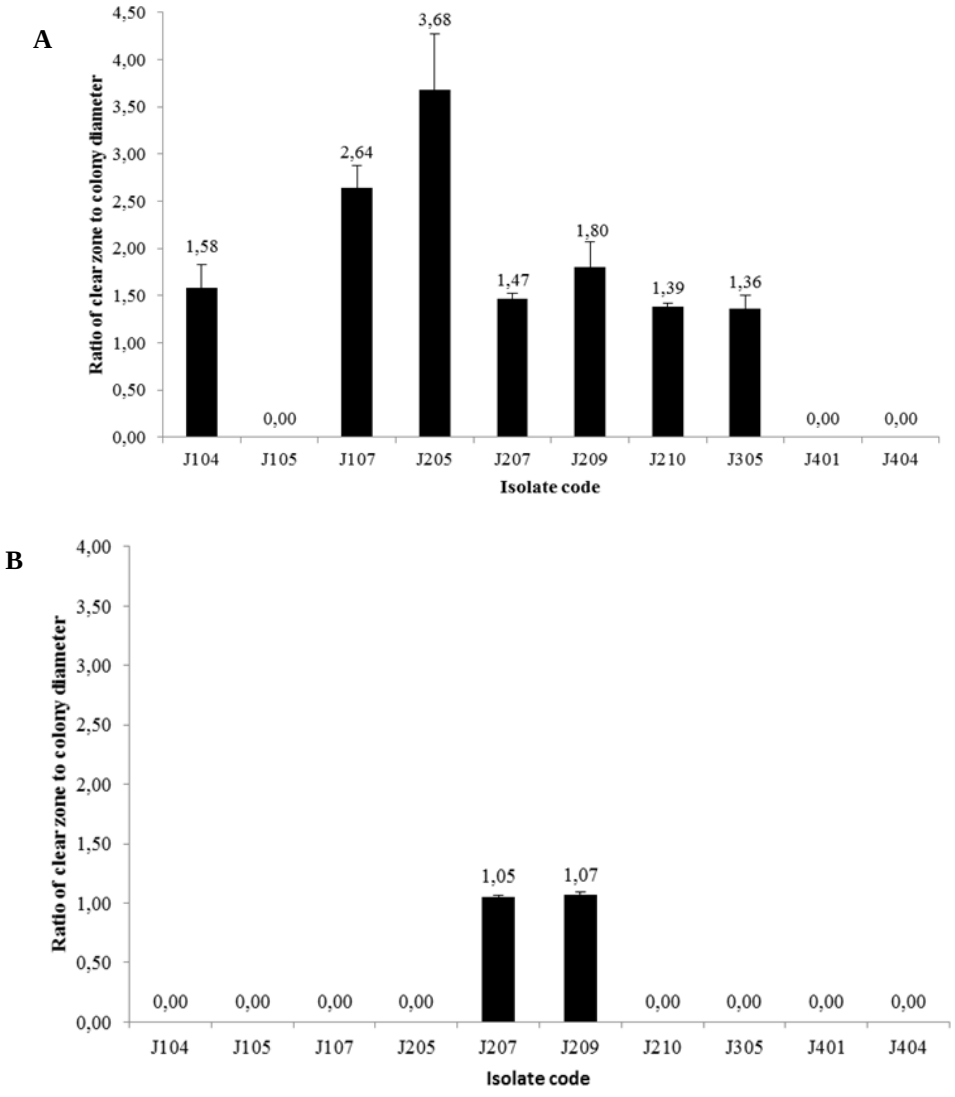

Figure 4. Synergistic test between cellulolytic bacteria isolates with *Staphylococcus saprophyticus* ICBB 9554 (A), and synergistic test between *Staphylococcus saprophyticus* ICBB 9554 with cellulolytic bacteria (B) using paper disc diffusion method, where the ratio of clear zone was calculated by divided the clear zone with colony diameter

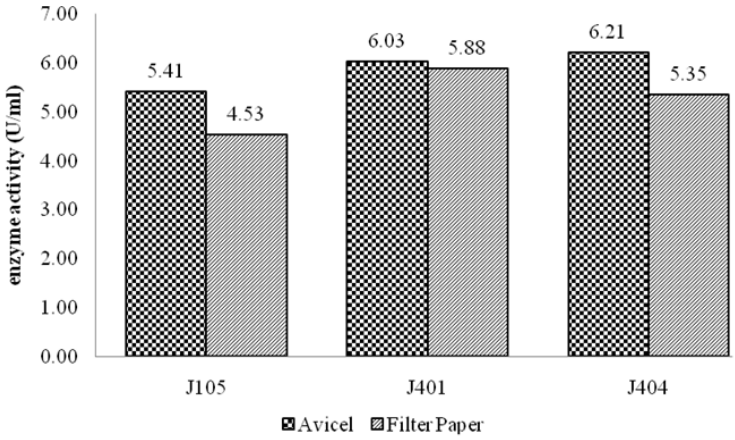

Figure 5. Enzyme activity assay of collected isolates using Avicel and filter paper as a substrate

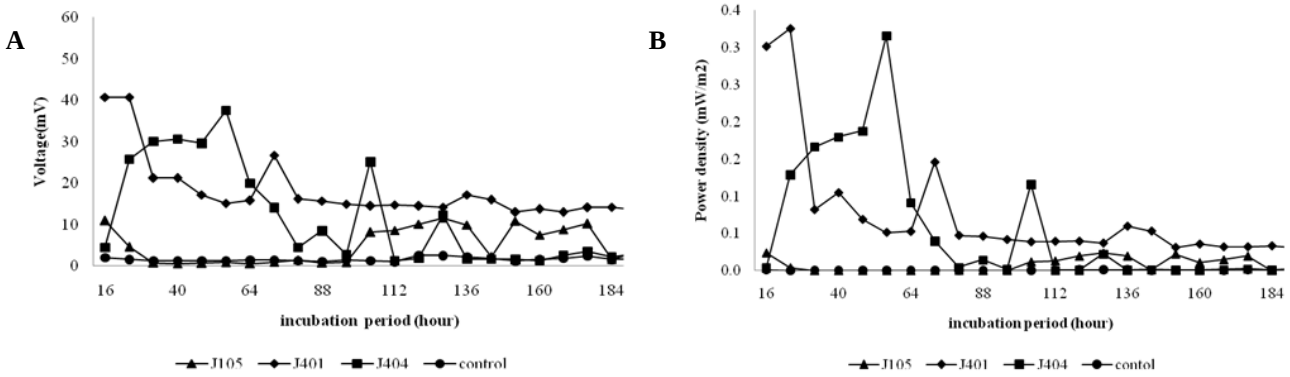

Figure 6. The voltage output as a function of time from the MFC with rice straw (0.3% w/v) as a substrate and catalyzed by different cellulose-degrading bacteria (A), power density (B) also as a function of time from the MFC

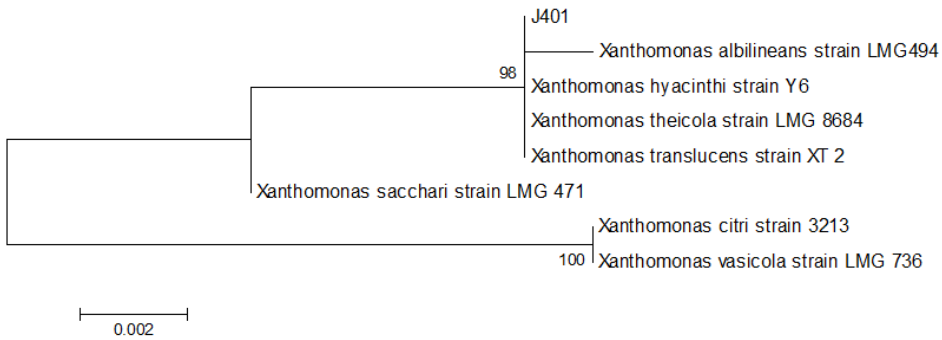

Figure 7. Phylogenetic position of the isolated strain J401; was shown using maximum likelihood with model/method of Kimura 2-parameter, and levels of bootstrap support percentage are indicated by the numbers at the nodes based on the maximum likelihood of 1000 replicates

Discussion

Lignocellulosic biomass contains main components in the form of cellulose (homopolysaccharides with β-1,4-glucan bonds), hemicellulose (heteropolysaccharides of xylans, mannans), and lignin (polyphenolic complex structures) (Perez et al. 2002). The quantity of each component varies and depends on several factors such as species, age of the plant, and which part of the plant was taken (Fengel and Wegener 1983). Cellulose is a major component of plant biomass and it is an abundant polymer on earth surface. Biodegradation and decomposition of cellulose biologically required the action of cellulose enzymes consisting of 3 components named endoglucanase, exoglucanase and β-glucosidase that work synergistically (Kostylev and Wilson 2012).

Cellulose degrading bacteria were isolated from rice straw with different decomposition levels: high, medium and low. The decomposition level was determined by the easiness of rice straw to break down upon squeezing. Staff Survey Soil (2014) divided the level of decomposition of plant material to become organic soil material in three categories based on fiber content, there was low (fibric), medium (hemic) and high (sapric). At a low level of decomposition, plant material contained up to 75% or more of fiber. When the level of plant decomposition was high, the fiber content decreases to below 17%. Plants with a moderate level of decomposition have a fiber content in the range between both low and high fiber content. Another parameter that reflects decomposition rate was C/N ratio. Decomposition process will decrease the C/N ratio. During decomposition process carbon will be mineralized as CO_2 or assimilated by the soil microflora, microbial decay-producing both C humification and secondary C mineralization. The N content improved due to a concentration effect caused by degradation of labile OC compounds which reduced the weight of the decomposting biomass (Thiyageshwari et al. 2017). The levels of decomposition of biomass can be influenced by several factors such as environmental physicochemical, biomass quality and content, and the composition of decomposers.

The ability of isolates to degrade cellulose in the Congo red method was proven by the existence of a colorless round zone that contrasted with a fixed red background on an undegradable substrate. CMC substrate became red when it was added by Congo red because Congo red provided a strong bond with polysaccharides (Teather and Wood 1982). Therefore, when the polysaccharide was successfully degraded, the red color will fade. The carboxymethylcellulose (CMC) was chosen as a substrate because CMC was soluble and readily hydrolyzed compared to crystalline cellulose for longer degraded (Eveleigh et al. 2009).

There were differences in the results of synergistic test using antagonism characteristic that was reflected by inhibitory zone of cellulolytic bacteria against *Staphylococcus saprophyticus* ICBB 9554 and *Staphylococcus saprophyticus* ICBB 9554 against cellulolytic bacteria. There were various assumptions of antagonistic mechanisms between different microorganisms. Long et al. (2013) reported that inhibition of growth of a microorganism by other microorganisms was due to substrate competition, local pH alternation, or the production of secondary metabolites. Other inhibitory mechanisms can be a combination of nutrition and space competition, antibiotic production, and resistance induction systems (Jiang et al. 2015). When several compounds from isolates are active either in crude or purified extracts, it is suggested that the majority of the interactions are true because of secondary metabolites (Teasdale and Donovan 2011). The secondary metabolites produced by some microorganisms such as antibiotics, siderophore, and lytic enzymes are used to inhibit the growth of other microorganisms. Then, the absence of inhibitory zone reflected synergism of cellulose-degrading bacteria with exoelectrogen bacteria. The production and secretion of organic acids by any microorganism is related to its nutrient supply and the corresponding metabolic activity. We assumed that cellulose-degrading bacteria which has synergistic relation with exoelectrogen bacteria-produced acid or supplied nutrient for exoelectrogen bacteria.

The conducted enzyme activity assay was the exoglucanase test with Avicel as a substrate and the total enzyme activity test with Whatman no 1. filter paper as a substrate. Avicel was chosen because it represented cellulose in the form of microcrystalline and the cell walls were composed of biopolymer interwoven tissue to form microcrystalline structures (Johnsen and Krause 2014). Whereas filter paper was chosen because it was easily available, inexpensive, and testing was able to provide results that could be repeated (Coward-Kelly et al. 2003). The filter paper Whatman No. 1 was a substrate for testing total cellulase activity according to procedures from the International Union of Pure and Applied Chemistry (IUPAC). In contrast to carboxymethylcellulose (CMC), both Avicel substrate and filter paper Whatman no 1. were insoluble cellulose substrates that were commonly used to test cellulase activity in hydrolyzing crystalline cellulose synergistically (Yu et al. 2016).

In Microbial Fuel Cells, exoelectrogen microbes have a role as catalysts and produce electricity directly. The exoelectrogen microbes are placed at the anode. The role of the catalyst is to oxidize organic material into electrons, protons, and carbon dioxide (Logan 2007). The MFC is a device that utilizes bacterial metabolism to produce electrical energy with a wide range of types of organic substrates. The organic material that has been tested on the MFC is cellulose. The conversion of cellulose into electricity requires a syntrophic consortium. The consortium will decompose complex substrates (cellulose) and produces acids that are used by exoelectrogen bacteria. Some researchers utilized cellulolytic degrading bacteria and exoelectrogen bacteria for producing electricity in MFC. This aimed to overcome the cost of chemical catalysts and the loss of electrons for soluble fermentation products. The bacterial consortium that works synergistically includes polymer decomposers, fermentation microorganisms, and microorganisms that utilize electrochemically active fermentation products (Ren et al. 2007). Then the difference result that the highest degenerators of cellulose are not the highest MFC output is assumed due to different acid that is produced during cellulolytic bacteria utilized cellulose. This assumption needs further analysis.

The best isolate which was identified based on sequence of 16S rRNA showed that the cellulose-degrading bacteria strain J401 had similar to *Xanthomonas translucens.* The J401 isolate has a yellow colony morphology and this was corresponding to Pruvost and Luisetti's (1989) description of the yellow-pigmented strains of *Xanthomonas* isolated from Brazil. The study produced information that *Xanthomonas* sp. *Mangiferae indicate* is biochemically able to hydrolyze cellulose. Behera et al. (2014) reported the research of the isolation of cellulose-degrading bacteria and one of the isolates was *Xanthomonas* sp.

In summary, rice is one of the agricultural crop plants with high productivity and high cellulose content. The rice straw can become waste so it needs to be managed properly. One option to manage rice straw waste is sped up the process of decomposition of rice straw. The decomposition can be accelerated by the presence of cellulose-degrading microorganisms. This research has succeeded in isolating some cellulose-degrading bacteria from rice straw with different levels of decomposition. Then the selected cellulose-degrading bacteria with high capability to decompose cellulose was tested to be a consortium that synergy with exoelectrogen bacteria, *Staphylococcus saprophyticus* ICBB 9554, in MFCs system. The result is one strain of cellulose-degrading bacteria, J401, has been selected as a candidate to have ability to synergy with exoelectrogen bacteria and identified as *Xanthomonas translucens.*

ACKNOWLEDGEMENTS

We would like to thank the Ministry of Research and Technology of the Republic of Indonesia through the PMDSU (*Program Mahasiswa Menuju Doktor untuk Sarjana Unggul*) scholarship (25/E/KPT/2018) for providing the funding for this research. We also thank Biology Laboratory of Soil Science and Land Resource Department of IPB University, Bogor, Indonesia which facilitated us for place and some equipment.

REFERENCES

Bayer EA, Lamed R, Himmel ME. 2007. The potential of cellulases and cellulosomes for cellulosic waste management. Curr Opini in Biotechnol 18 (3): 237-245.

Behera BC, Parida S, Dutta SK, Thatoi HN. 2014. Isolation and identification of cellulose-degrading bacteria from mangrove soil of Mahanadi river delta and their cellulase production ability. Amer J Microbiol Res 2 (1): 41-46.

Bhat MK, Bhat S. 1997. Cellulose degrading enzymes and their potential industrial application. Biotechnol Adv 15 (3): 583-620.

Chantarasiri A. 2015. Aquatic *Bacillus cereus* JD0404 isolated from the muddy sediments of mangrove swamps in Thailand and characterization of its cellulolytic activity. Egyp J Aquat Res 41: 257-264.

Coward-Kelly G, Aiello-Mazzari C, Kim S, Granda C, Holtzapple M. 2003. Suggested improvements to the standard filter paper assay used to measure cellulase activity. Biotechnol Bioeng 82: 745-749.

Dantur KI, Enrique R, Welin B, Castagnaro AP. 2015. Isolation of cellulolytic bacteria from the intestine of *Diatraea saccharalis* larvae and evaluation of their capacity to degrade sugarcane biomass. AMB Exp 5 (15): 1-11.

Eveleigh DE, Mandels M, Andreotti R, Roche C. 2009. Measurement of saccharifying cellulose. Biotechnol Biofuel 2 (21): 1-8.

FAO. 2018. Rice market moitor. Round Up 21 (1): 1-38.

FAO. 2019. Standard operating procedure for soil organic carbon: Walkley-Black method Titration and colorimetric method. Glosolan SOP Tech. W. G. Philippines.

Fengel D, Wegener G. 1983. Wood: chemistry, ultrastructure, reactions. Walter de Gruyter, Berlin.

Ghose TK. 1987. Measurement of cellulase activities. Pure Appl Chem. 59 (2): 257-268.

Gupta P, Samant K, Sahur A. 2012. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. Intl J Microbiol 1: 1-5.

Huang L, Logan BE. 2008. Electricity generation and treatment of paper recycling wastewater using a microbial fuel cell. Appl Microbiol Biotechnol 80: 349-355.

Huang S, Sheng P, Zhang H. 2012. Isolation and Identification of Cellulolytic Bacteria from the Gut of *Holotrichia parallela* Larvae (Coleoptera: Scarabaeidae). Intl J Mol Sci 13: 2563-2577.

Jiang CH, Wu F, Yu ZY, et al. 2015. Study on screening and antagonistic mechanisms of *Bacillus amyloliquefaciens* 54 against bacterial fruit blotch (BFB) caused *by Acidovorax avenae* subsp. Citrulli J Micres 170: 95-10.

Johnsen HR, Krause K. 2014. Cellulase Activity Screening Using Pure Carboxymethylcellulose: Application to Soluble Cellulolytic Samples and to Plant Tissue Prints. Intl J Mol Sci 15: 830-838.

Jusoh MLC, Manaf LA, Latif PA. 2013. Composting of rice straw with effective microorganisms (EM) and its influence on compost quality. Iranian J Environ Health Sci Eng 10 (17): 1-9

Kawale HD, Renveer AC, Chavan AR. 2017. Electricity generation from wastewater using a microbial fuel cell by using mixed bacterial culture. J Biochem Tech 8 (2): 1123-1127.

Khawdas W, Watanabe K, Karatani H, Aso Y, Tanaka T, Ohara H. 2017. Direct electron transfer of *Cellulomonas fimi* and microbial fuel cells fueled by cellulose. J Biosci Bioeng 1-6.

Kjeldahl JZ. 1883. A new method for the determination of nitrogen in organic bodies. Zeitschrift für Analytische Chemie 22 (1): 366-382

Kostylev M, Wilson D. 2012. Synergistic interactions in cellulose hydrolysis. Biofuels 3 (1): 61-70

Liang YL, Zhang Z, Wu M, Wu Y, Feng JX. 2014. Isolation, Screening, and Identification of Cellulolytic Bacteria from Natural Reserves in the Subtropical Region of China and Optimization of Cellulase Production by *Paenibacillus terrae* ME27-1. Biomed Res Intl 2014: 512497. DOI: 10.1155/2014/512497

Logan BE, Regan JM. 2006. Microbial fuel cells-challenges and applications. Environ Sci Technol 40: 5172-5180.

Logan BE. 2007. Microbial Fuel Cell. Wiley, New Jersey.

Long RA, Eeillard D, Franco SLM, Reeves E, Pinkney JL. 2012. Antagonistic interactions between heterotrophic bacteria as a potential regulator of community structure of hypersaline microbial mats. FEMS Microbiol Ecol 83: 74-81.

Mandels M, Reese ET. 1957. Induction of cellulase in *Trichoderma viride* as influenced by carbon sources and metals. J Bacteriol 73 (2): 269-278.

Mazzapioda L, Panero S, Navara MA. 2019. Polymer electrolyte membranes based on Nafion and a super acidic inorganic additive for fuel cell applications. Polymers 11: 1-10.

Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31 (4): 426-428.

Parker N, Schneegurt M, Tu AHT, Forster BM, Lister P. 2017. Microbiology. Openstax, Texas.

Perez J, Munoz DJ, Rubia T, Martínez J. 2002. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. Intl Microbiol 5: 53-63.

Peristiwati, Natamihardja YS, Herlini H. 2018. Isolation and identification of cellulolytic bacteria from termites gut (*Cryptotermes sp.*). J Physics Conf Series 1013: 012173. DOI: 10.1088/1742-6596/1013/1/012173

Pruvost O, Luisetti J. 1989. Strains of *Xanthomonas campestris* isolated from Ambarella (*Spondias cytherea* Sonn.) in the French Reclassi West Indies are probably a new pathogenic form of *Xanthomonas campestris* pv. *mangiferaeindicae*. Fruits 44: 539-542.

Rawway M, Ali SG, Badawy AS. 2018. Isolation and identification of cellulose-degrading bacteria from different sources at Assiut Governorate (Upper Egypt). J Ecol Heal Env 6 (1): 15-24.

Razaei F, Xing D, Wagner R, Regan JM, Richard TL, Logan BE. 2009. Simultaneous cellulose degradation and electricity production by *Enterobacter cloacae* in a microbial fuel cell. Appl Environ Microbiol 75 (11): 3673-3678.

Ren Z, Ward TE, Regan JM. 2007. Electricity production from cellulose in a microbial fuel cell using a defined binary culture. Environ Sci Technol 41: 4781-4786.

Sáez-Plaza P, Navas MJ, Wybraniec S, Michałowski T, Asuero AG. 2013. An overview of the Kjeldahl method of nitrogen determination. Part II. Sample preparation, working scale, instrumental finish, and quality control. Crit Rev Amalyt Chem 43: 224-272.

Saha S, Roy R, Sen SK, Ray AK. 2006. Characterization of cellulose producing bacteria from the digestive tract of tilapia, *Oreochromis mossambica* (Peters) and grass carp, *Ctenopharyn godonidella* (Valenciennes). Aquacult Res 37: 380-388.

Shil RK, Mojunder S, Sadida FF, Uddin M, Sikdar D. 2014. Isolation and identification of cellulolytic bacteria from the gut of three phytophagous insect species. Braz Arch Biol Technol 57 (6): 927-932.

Singh RB, Soha RC, Singh M, et al. 1995. Rice Straw-Its Production and Utilization in India. Handbook for Straw Feeding Systems. ICAR, New Delhi, India.

Soares-Junior FL, Dias ACF, Fasanella CC, et al. 2013. Endo-and exoglucanase activities in bacteria from mangrove sediment. Brazil J Microbiol 44 (3): 969-976.

Soil Survey Staff. 2014. Keys to Soil Taxonomy. 12th ed. NRCS-USDA, Washington DC.

Teasdale ME, Donovan KA, Forschner-Dancause SR, Rowley DC. 2011Gram-positive marine bacteria as a potential resource for the discovery of quorum sensing inhibitors. Mar Biotechnol 13: 722-732.

Teather RM, Wood PJ. 1982. Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl Environ Microbiol 43 (4): 777-780.

Thiyageshwari S, Gayathri P, Krishnamoorthy R, Anandham R, Paul D. 2017. Exploration of rice husk compost as an alternate organic manure to enhance the productivity of black gram in *Typic Haplustalf* and *Typic Rhodustalf*. Intl J Environ Res Public Health 15 (358): 1-14.

Thygesen A, Poulsen FW, Angelidaki I, Min B, Bjerre AB. 2011. Electricity generation by microbial fuel cells fuelled with wheat straw hydrolysate. Biomass Bioenerg 35: 4732-4739.

Upadhyaya SK, Manandhar A, Mainali H, et al. 2012. Isolation and characterization of cellulolytic bacteria from gut of termite. Rent Sympos Compen 1: 14-18.

Venkadesan D, Sumathi V. 2015. Screening of lactic acid bacteria for their antibacterial activity against milk-borne pathogens. Intl J Appl Res 1 (11): 970-973.

Waghmare PR, Patil SM, Jadhav SL, Jeon BH, Govindwar SP. 2018. Utilization of agricultural waste biomass by cellulolytic isolate Enterobacter sp. SUK-Bio. Agricult Natur Resour 52: 399-406.

Walkley A, IA Black. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci 37: 29-37.

Yu X, Liu Y, Cui Y. 2016. Measurement of filter paper activities of cellulose with microplate-based assay. Saudi J Biolog Sci 23: 93-98.