



Research Paper

Cellulose degradation and electric flux enhancement power of indigenous bacteria from kitchen waste

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ABSTRACT

Kitchen waste, containing a huge amount of organic waste, is a source of energy for microbes. The present study was conducted to determine the ability of bacteria to degrade organic substrate and show their electric flux generation potential from kitchen waste. Twenty six bacteria, (KW1 to KW26) were isolated from kitchen waste. Only five bacterial isolates, KW8, KW14, KW16, KW22 and KW24, showed ability to degrade cellulose. The highest cellulase activity was shown by isolate KW16 6.84 ± 0.3 IUmL⁻¹, followed by KW14 6.09 ± 0.2 IUmL⁻¹ at 72 h. Only KW16 showed highest electricity generation of 81.9 ± 0.9 mV. These isolates has oxidoreductase enzymes that participate in enhancing electric current generation mechanism in the use of kitchen waste residue. The microbial ability for waste to generate electric current can provide a platform to design a sustainable bioreactor based conversion technology which can increase the source of bioenergy.

Yogita Basene and Ragini Gothalwal*

Department of Biotechnology,
Barkatullah University Bhopal (MP),
462026, India.

*Corresponding author. E-mail address:
ragini_gothalwal@yahoo.com,
yogita.basene@gmail.com.

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INTRODUCTION

The domestic waste contains huge amount of kitchen waste and is composed of starch, protein and cellulose which are organic in nature. Organic waste contains high moisture and high salinity (Meng et al., 2015), and health problems could increase due to environmental pollution by this substance (Shen et al., 2013). Conventional methods are not significant for decomposing the organic material, due to the loss of biodegradable waste that can be a sustainable source of energy (Grimberg et al., 2015; Huang et al., 2015). Cellulolytic microbes have an ability to degrade cellulosic material present in kitchen waste into other useful products (Nathalie, 2009). These microbes are beneficial due to their degrading ability by reducing large and complex molecules into different valuable products, such as simple sugar, biomass protein, compost and generate biofuels (Sanchez and Cardona, 2008), and form of bioenergy (Li et al., 2009). The microbes produce the extracellular enzyme such as amylases, proteinases and polysaccharides hydrolases (Mawadza et al., 2000), which can participate in the biodegradation of organic waste. For example cellular enzyme systems are the major domain of

the microbial enzyme system, which can hydrolyze cellulose thereby forming glucose and other artifacts (Chandra et al., 2009). Cellulase can be used to convert cellulose into simple sugars. Cellulase is produced by a wide range of organisms including bacteria, fungi, and invertebrates (Gautam et al., 2011). Various bacterial strains, belonging to the phyla Firmicutes, Actinobacter, Proteobacteria and Bacteroidetes (Koeck et al., 2014; Sukharnikov et al., 2011), are capable of degrading cellulose (Himmel et al., 2010 ; Větrovský et al., 2014; Yang et al., 2014). A group of gram positive and gram negative species have the ability to produce cellulase, for example *Bacillus subtilis*, *Cellulomonas* sp., *Clostridium thermocellum*, *Proteus*, *Pseudomonas* sp., *Ruminococcus* sp., *Serratia*, *Staphylococcus* spp. and *Streptomyces* sp. (Wood and Bhat 1982; Gautam et al., 2010). Cellulase is part of the carbohydrate active enzymes (CAZymes), with catalytic and carbohydrate-binding modules (or functional domains) that degrade or create glycosidic bonds (Lombard et al., 2014). Cellulase is divided into three classes of soluble extracellular enzymes: 1, 4-β-endoglucanase, 1, 4-β exoglucanase and β-

glucosidase (β -D-glucoside glucohydrolase or cellobiase participate in this simple sugar/protein activity) (Singhania et al., 2010). Organic waste contains huge amount of high molecular weight substances such as cellulose, protein and lipids, which could be easily degraded by microbes. Microbial fuel cell is a good method to utilized organic matter of the waste to generated electricity (Jia et al., 2013; Moqsud et al., 2014). Simple organic compound is used by microbes for their metabolic activity. Some enzymes such as ubiquinone, NADH dehydrogenase, and cytochrome participate in the oxidative metabolic pathway of microorganisms and participate in oxidation-reduction reaction (Bretschger et al., 2007; Goa et al., 2010). Electron and proton will be transferred through these particular enzymes and produce electric flux during these processes (Lovley, 2008). In this study, kitchen waste was collected and used for the isolation and screening of bacteria with the potential to degrade organic waste such as cellulose for the purpose of electric flux generation during microbial fuel cell operation.

MATERIALS AND METHODS

Sample collection

The sample was collected directly from waste in baskets or dustbin of kitchens from different places in replicates. Waste was collected into pre - sterilized screw cap glass vials of 15 ml capacity's, and selected 2 cm² blocks of waste pieces were scrapped with spatula and collected in vials for isolation of cellulose degrading bacteria.

Isolation of bacteria from kitchen waste

Serial dilution and streaking plate method were done to obtain pure bacterial isolates using of Nutrient Agar Medium (NAM) (Cappuccino and Sherman, 1996).

The cellulolytic ability of bacterial isolates was determined using the enrichment technique through the Busnell Hass Medium (BHM) (Singh et al., 2013), the composition was as follows (gL⁻¹): K₂HPO₄ - 1.0, KH₂PO₄ - 1.0, MgSO₄.7H₂O - 0.2., NH₄NO₃ - 1.0, FeCl₃.6H₂O - 0.05, CaCl₂ -0.02 and Agar-15.0, Carboxymethyl cellulose (CMC) (Molecular weight -262.19) of - 5.0 was used as substrate with pH 7.0 \pm 0.2.

Screening of cellulose degrading bacteria

Cellulose degrading bacterial isolates were screened on the basis of two suitable enzyme assays. The first involves the qualitative screening of cellulolytic bacterial by the plate staining method (Teather and Wood, 1982; Singh et al., 2013) and the second is the quantitative enzyme assay

performed to determine the amount of reducing sugar liberated using dinitrosalicylic (DNS) method (Miller, 1959). The BHM medium was used for both quantitative and qualitative estimation.

Congo red plate assay

For qualitative estimation - Firstly, culture medium plate was prepared and fresh bacterial culture of individual isolates (loopful) were inoculated into the solidified plates and then incubated at 37°C for 96 h. Then plates were flooded with 0.2% congo red solution used as an indicator and kept for 20 min. The stain was poured off, and the plates were washed with 1M NaCl. Thereafter, the zone of clearance and colony diameter were measured. Cellulose degrading characteristics of the potential isolates was measured by calculating the hydrolysis capacity (HC) of carboxymethyl cellulose utilized; HC is the ratio of diameter of the zone of clearance and colony (Hendricks et al., 1995; Gupta et al., 2012). The maximum hydrolytic capacities of bacterial isolates were selected for further estimation of cellulase.

Cellulase enzyme production and determination

The enzyme production media was prepared with 0.5% CMC in BHM broth. The 0.5 ml of 24 h fresh bacterial culture was inoculated into 50 ml freshly prepared medium and incubated at 37°C with 150 rpm shaking. After 2 days of incubation, the broth was centrifuged at 8000 rpm for 20 min. Then the supernatant was collected for the activity of cellulase in the culture filtrate. The reaction mixture with 1.0 ml of 0.5% CMC in 0.2 M acetate buffer (pH5.0) was incubated at 50°C in water bath for 20min. An aliquot of 1 ml of culture filtrate with appropriate amount was added to the reaction mixture and incubated at 50°C in waterbath for 1 h. The reducing sugar produced in the reaction mixture was determined using DNS method (Miller, 1959), where 3, 5 Dinitrosalicylic acid reagents were added to aliquots of the reaction mixture and the color developed was recorded at 540 nm.

Potential for electric flux generation in bacterial isolates

This ability of cellulolytic bacterial was tested by the microbial fuel cell assembly. Microbial fuel cell was prepared through various components, such as polypropylene, in 250 ml capacity bottles used for the anode and cathode chamber. Chambers were connected with salt bridge prepared by the plastic pipe with 7 cm \times 1.0 cm, and bridge was filled with KCl + Agar (2.5% KCl and 3% agar). Carbon paper (4.0 cm length and 1.5 cm width) was

used for the electrode preparation, and copper wire was used to connect the electrodes inside the chambers.

Microbial fuel cell operation

The anode chamber was filled and autoclaved with 150 ml of BHM medium, where 0.5% CMC was added as substrate and inoculated for 24 h with 1 ml of freshly prepared bacteria culture (OD 0.220) maintained anaerobic condition. The cathode chamber, where oxygen was used as the electron acceptor for the electrode, was filled with 100 mM phosphate buffer; pH 7.0 ± 0.2 was maintained. The cathode chambered was connected with air pump that provided air.

Analyses of output

All experiments were conducted at the room temperature and incubated for 3 days. For power output, a range of resistance (0.1 to 1.0 Ω) was used as external load. The voltage was recorded through the digital multimeter at 24 h intervals up to three days.

Statistical analysis

All experiments were performed in triplets. The data of all the parameters of isolates were statistically analyzed using Microsoft excel - XLSTAT 2010. The data for plate assay, enzyme activity and electric flux generation were analyzed at $p < 0.05$ by performing one way ANOVA, and least significant difference was calculated by student's t- test.

Identification of bacterial isolates

Cellulose degrading and electric flux generation potential of bacterial isolates were identified on the basis of morphology and biochemical characterization in Bergy's manual (Cappuccino and Sherman, 2005).

RESULTS AND DISCUSSION

Cellulose degrading and electric flux producing bacteria were isolated from kitchen waste using serial dilution and spread plate technique on NAM plate. A total of 26 bacteria (KW1 to KW26) were isolated. All the bacterial isolates were purified on BHM medium.

Cellulolytic activity in plate assay

Among the 26 isolates, only thirteen bacterial isolates

(KW1, KW3, KW5, KW8, KW14, KW16, KW17, KW19, KW21, KW22, KW24, KW25 and KW26) showed positive cellulolytic activity on the CMC agar plate and congo-red staining assay method. The maximum CMC hydrolytic capacity occurred in KW16 4.63 ± 0.23 mm, followed by KW14 4.4 ± 0.2 mm and KW8 4.2 ± 0.19 mm after 96 h of incubation as compared with other bacterial isolates (Figure 1).

Lu et al. (2006) reported that the mesophilic cellulose degrading bacteria obtained from vegetables waste showed similar range of hydrolytic capacity. Hatami et al. (2008) observed that cellulolytic aerobic bacterial isolated from farm showed hydrolytic value of between 1.38 to 2.33 and 0.15 to 1.37 in the case of forest soil bacteria. While Gupta et al. (2012) reported that hydrolytic capacity range was 4 to 9 mm of the cellulolytic bacteria isolated from the invertebrates.

The maximum hydrolytic capacity obtained (> 3 mm) by 7 bacterial isolates (KW1, KW8, KW14, KW16, KW17, KW22, KW24) were selected to study their growth curve and quantitative enzyme activity. This indicates that potent isolates could produce cellulase enzyme with maximum cellulolytic activities, which might have the some active protein molecules that increase the potential level for cellulose degradation. Congo red plate assay method can be beneficial for the screening of the cellulase enzyme producing bacteria. The present study obtained a bacterial isolate KW14 and KW16 from the kitchen waste, which have a great efficacy to convert organic waste into simple molecules due to the presence of cellulase enzyme.

Growth curve and enzyme activity

The growth pattern of potential six bacterial isolates is shown in Figure 2. The isolate KW16 attained maximum growth (OD. 1.97) at 72 h of incubation during enzyme production. The absorbance declined to 1.560 at 96 h of incubation (Figure 2).

The isolate KW14 also indicated maximum growth (OD. 1.73) at 72 h of incubation. The isolate KW16, showed maximum growth, maximum cellulase production and maximum current generation at 72 h of incubation. This result indicated that cellulase production and current generation is directly proportional to the bacterial growth or number of bacterial cells. Similar finding has been reported by Khatiwada et al. (2016) on the correlation of cellulase production with growth. The *Bacillus* and *Serratia* isolates showed maximum level of cellulase production after 24 h of cultivation and maximum colony forming unit was observed as well.

The cellulase activity was determined by quantitative assay, where maximum enzyme secretion was shown by isolate KW16 6.84 ± 0.3 IUmL⁻¹, KW14 6.09 ± 0.2 IUmL⁻¹ and KW8 5.03 ± 0.4 IUmL⁻¹ at 72 h of enzyme production (Figure 3).

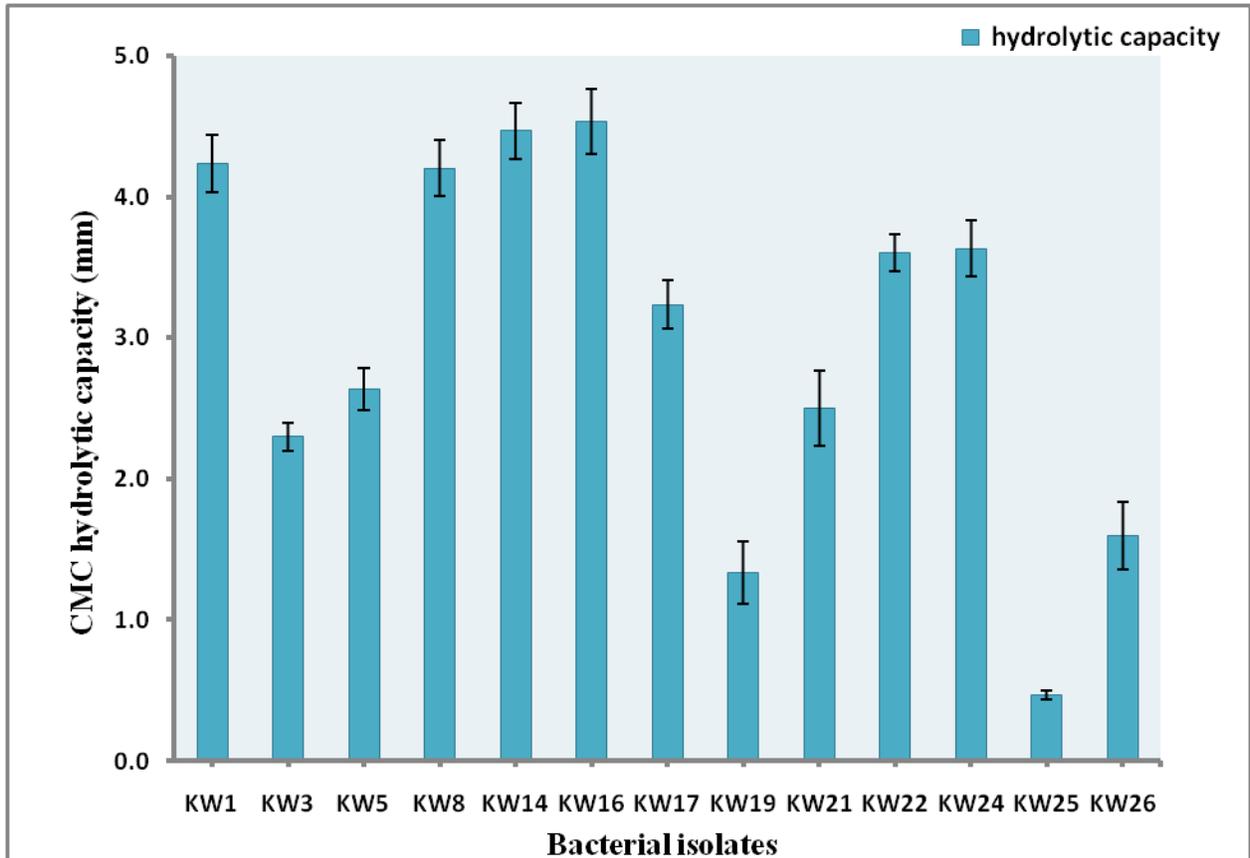


Figure 1: Screening of the cellulase producing bacteria through the congored plate assay indicated the zone of clearances (cm) around the colony at 96hr of incubation. Values are given into Mean \pm SD.

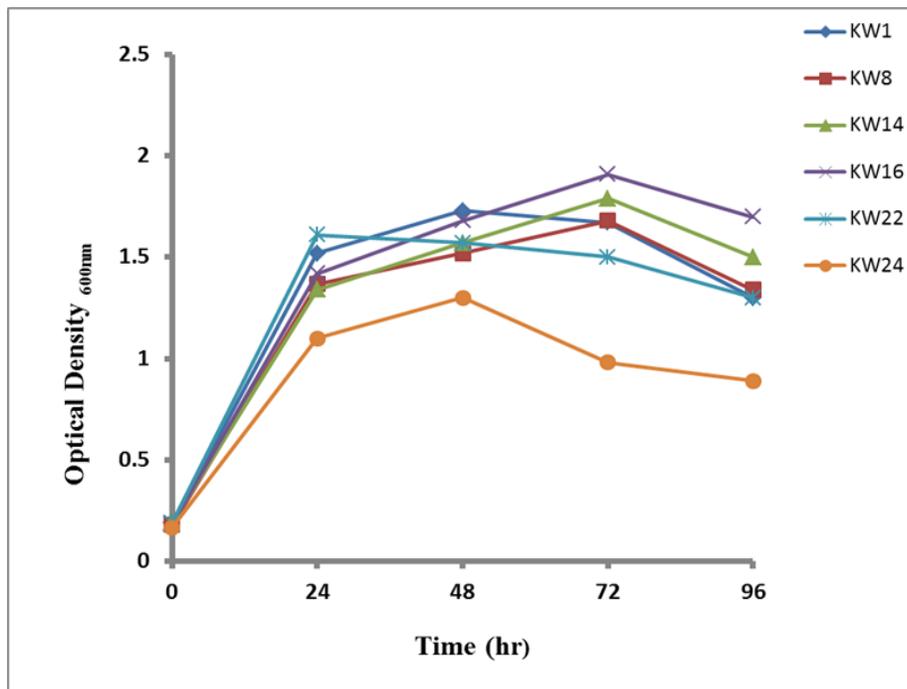


Figure 2: Growth patterns of the cellulolytic bacterial isolates in enzyme production medium (0.5% CMC as substrate).

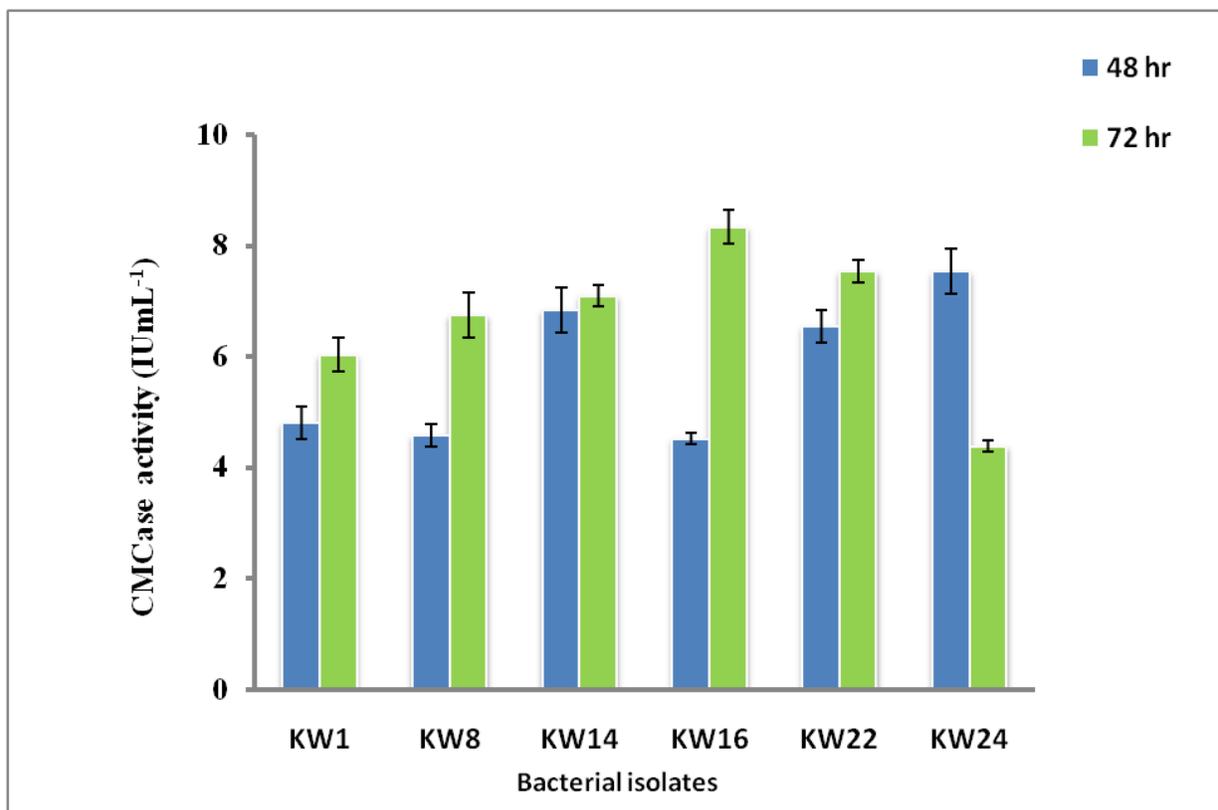


Figure 3: Cellulase activity of potent bacterial isolates was determined by DNSA method. Measurements were performed in triplicate and standard bars response the standard deviation.

Kumar et al. (2012), in their study, reported cellulase activity of 66 U mL^{-1} for *Bacillus cereus*, and this is similar to enzyme activity of KW16 and KW14. This indicates that these bacteria may belong to *Bacillus* spp. and this result obtained may be due to the presence of extracellular enzyme. According to Molva et al. (2009), *Bacillus thuringiensis* strain was found to produce extracellular enzyme.

Liang et al. (2014) showed that *Paenibacillus terrae* ME 27-1 showed the highest CMCase activity (0.17 U mL^{-1}) after incubation for 60 h in presence of 1% CMC. *Bacillus subtilis* AS3 used CMC as carbon source and showed only 0.43 U mL^{-1} enzyme activities (Deka et al., 2011). Maki et al. (2011) indicated that *Paenibacillus* showed total cellulase activity of $1652.2 \pm 6.5 \mu\text{M}$ with CMC (1% w/v). Moreover, Singh et al. (2013) reported that *Bacillus* spp. exhibited maximum cellulase enzyme activity of 0.079 U mL^{-1} in the presence of CMC. This indicates that *Bacillus* spp. have an ability to convert cellulose such as complex compound into glucose. These molecules act as a beneficial source for the electric flux generation.

Electric flux detection

On the basis of both quality and quantitative cellulase

detection, four bacterial isolates KW8, KW14, KW16 and KW24, were selected for further electric flux generation potential. In KW16, the highest electric flux generating potential was found to be $81.9 \pm 0.9 \text{ mV}$ on the 3rd day operations/incubation of microbial fuel cell, followed by the KW with 1477.4 ± 1.3 on the 3rd day of incubation. KW8 was shown comparatively to induce less current of $40.5 \pm 1.2 \text{ mV}$ on the 3rd day of operation (Figure 4)

In present study, KW16 produced maximum electric potential of $81.9 \pm 0.25 \text{ mV}$ in on the 3rd day of incubation of MFC operation in presence of 0.5% CMC as carbon source. In a study, Rezaei et al. (2009) expressed that the *E. cloacae* FR produced $4.9 \pm 0.01 \text{ mW/m}^2$ power density in U-tube MFC and cellulose as a substrate, without exogenous mediators. This shows the possibility of simultaneous cellulose degradation and electricity production. Chen et al. (2017) reported that phylum proteobacteria were dominant in the microbial fuel cell (MFC), such as *Bacillus subtilis*, *Flavobacterium* sp., *Aeromonas hydrophilia*, *Citrobacter freundii*, and *Stenotrophomonas* spp., and they have potential for electricity generation. Food waste and organic waste from cafeterias and canteen have been used to produce electricity as a total food waste of 1000 kg day^{-1} was used to generate electricity of approximately 600 KW (Mydin et al., 2014). This indicates that these wastes are sources of energy. Li et al. (2016) reported that food waste

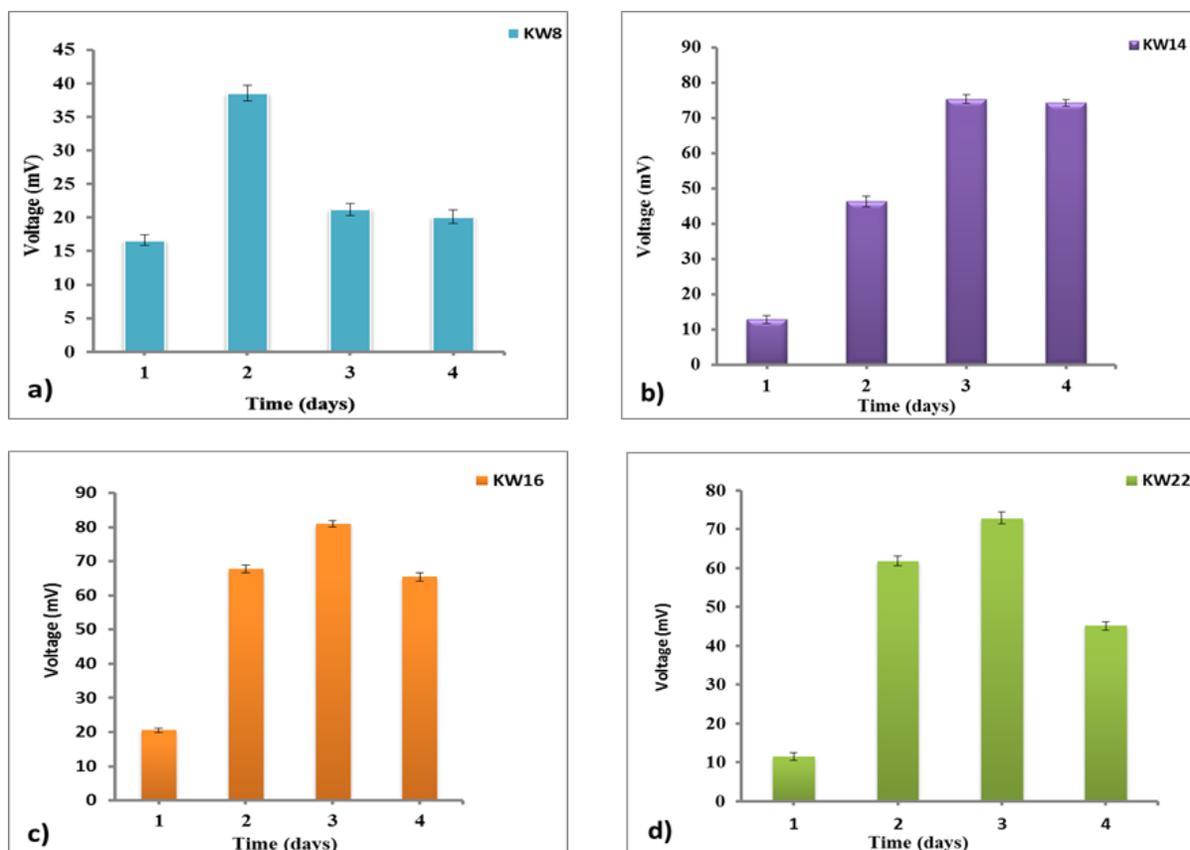


Figure 4: Electric flux generation of the potential isolates a) KW8 b) KW14 c) KW16 d) KW22 at maximum 4 days operation of the microbial fuel cell. Values in the figure are means of three replicates with standard deviation.

containing organic matter was used during the MFC treatment and average output voltage of 0.51V was obtained. Ren et al. (2008) showed a power density of 153 mW/m² using carboxy methyl cellulose as a substrate. Further, Kim et al. (2009) reported that the bacterial expanse from *B. subtilis* enhanced enzymatic hydrolysis of cellulose. The results of previous studies are in line findings of the present study. The isolate KW16 belonged to *Bacillus* spp. and showed the ability to degrade cellulose and as such, can be a good electric current enhancer. This KW16 contain a powerful oxidoreductase enzymes such as oxidase or dehydrogenase, which participate in the current generation mechanism.

Identification of bacterial isolates

The identified potent organisms found on the electrode surface during MFC operation were evaluated to determine their morphology characteristic. Morphological characteristics revealed that all four isolates KW8, KW14, KW16 were rod shaped and KW24 cocci in structure and gram staining results showed that KW8 and KW24 were gram negative, apart from this KW14 and KW16 which were gram positive (Figure 5 and Table 1).

Biochemical properties, such as starch hydrolysis, nitrate, catalase, and urease test, were further examined in these isolates (Table 2), where all isolates were urease negative, catalase positive was shown by KW8, KW16, KW24 and only KW14 showed catalase negative test. The isolate KW16 showed positive in starch hydrolysis test.

This test indicated that the isolates belong to the *Bacillus* group. These characteristics indicated that the organisms are *Bacillus* spp., according to the Cappuccino and Sherman (2005).

Priest (1977) reported the genus *Bacillus* had characteristics of extracellular amylase enzyme production and this enzyme participated in starch hydrolysis process. Moreover, Kim et al. (2012) reported that cellulolytic bacterial isolates showed the production pattern of cellulose degrading enzyme and were identified as *B. subtilis* strain by morphological, biochemical and 16sRNA gene analysis.

Conclusion

This study concluded that the potent bacteria obtained from kitchen waste showed dual characteristics. These bacterial isolates showed the property of cellulosic

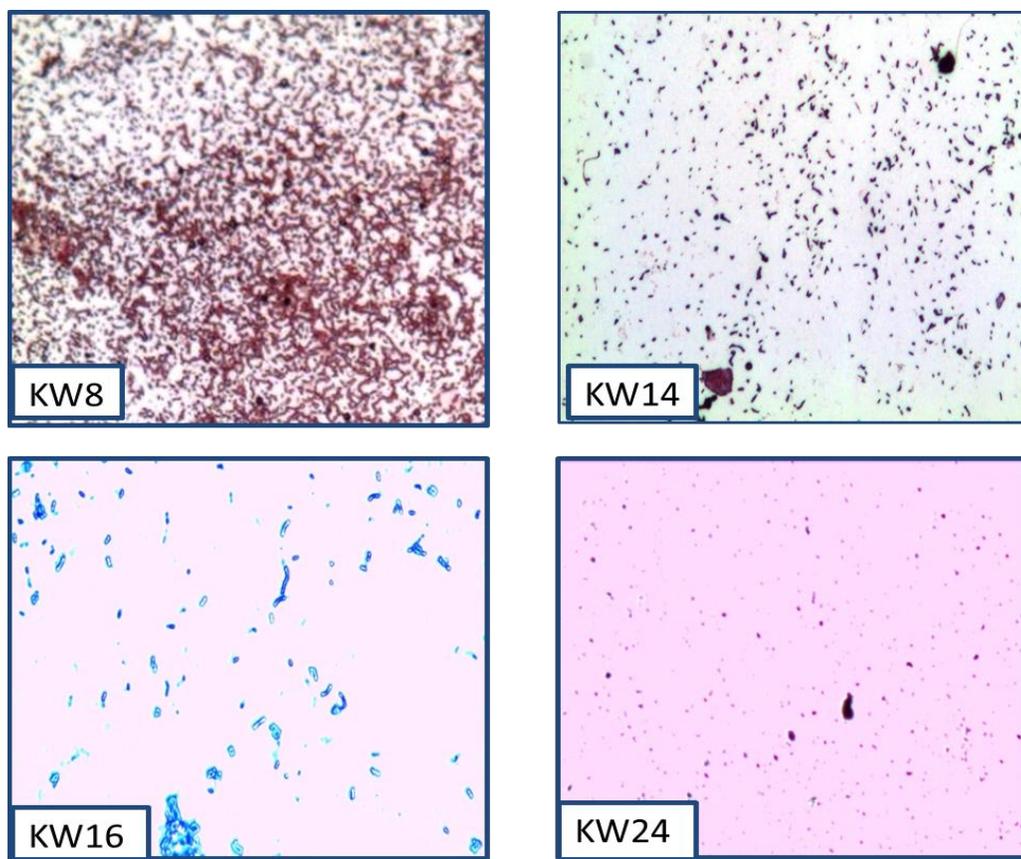


Figure 5: Microphotograph of the potent bacterial isolates of the gram staining (40X).

Table 1: Morphological characteristics of the bacterial isolates.

Isolates codes	Colony morphology	Cell morphology	Grams stain
KW8	Yellow, large, irregular, flat, dry, dull, translucent	Rod	Negative
KW14	Whitish, rhizoid, dry, small, flat, opaque	Rod	Positive
KW16	White, round, smooth, mucoid, short chain, opaque	Rod	Positive
KW24	White, pinpoint, round, dry	Cocci	Negative

+, positive reaction; -, negative reaction

Table 2: Biochemical characteristics of the bacterial isolates

Test Performed	KW8	KW14	KW16	KW24
Indole test	-	-	-	-
Methyl red test	-	-	-	-
Voges Proskauer test	-	-	-	-
Citrate utilization	+	-	+	-
Starch hydrolysis	-	-	+	+
Urea hydrolysis	-	-	-	-
Nitrate reduction	-	-	+	-
H ₂ S production	-	-	-	-
Catalase test	+	-	+	+
Oxidase test	+	-	-	-

+, positive reaction; -, negative reaction

substrate degradation and electric flux generation simultaneously in very cost-effective and environmentally safe microbial fuel cell. The bacterial isolates KW16 and KW14 can be further used for the large amount of electricity production through bioreactor design technology for waste treatment plants. Therefore, further molecular characterization and phylogenetic study is required on the identification of bacteria species. This could help to identify the diversity of current producing bacteria. The bacteria from kitchen waste can play a key role in electricity generation and part of the attractive process for bioenergy production.

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