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Utilization of Putative *Enterobacter* Isolate and Substrates for Microbial Fuel Cells

ABSTRACT

The current Philippine energy crisis reminds us of the importance of finding alternative energy sources. Microbial fuel cells (MFC) may contribute to the solution. MFCs utilizing marine sediments, rice straw, domestic sewage, and agricultural water have a large potential as an alternative energy source. The objectives of the project were to isolate the biological agent, determine the optimum waste substrates, and to develop a working microbial fuel cell using locally available materials as fuel source. Soil, sediment, and corn stover were collected. An improvised MFC was constructed with two compartments for the anode and cathode sections separated by an agar plug (5% w/v). Each compartment had 750 ml capacities. Several combinations of materials were determined. Triplicates of each material-isolate combination were used to determine voltage, amperage, and Columbic output. Thirty percent fish farm sediments produced the highest voltage and amperage. This treatment was able to produce power for 7 to 25 days after MFC setup. Addition of ammonium sulfate in this setup reduced electrical output. Other treatments also produced power but were not as comparable. This study showed that utilizing wastes as substrate for MFCs is feasible and may have practical use.

Key words: *Microbial fuel cell, Enterobacter cloacae, waste utilization*

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INTRODUCTION

The recent energy crisis of the Philippines has reminded us of the importance of finding alternative energy sources. However, resource utilization should be done while considering the environment. Microbial fuel cells (MFC) may contribute to the answer. Waste products such as corn stover, rice straw, domestic sewage, and refuse have a large potential as alternative energy sources. Rather than discarding these materials, converting these resources into usable energy via MFCs will help save the environment and better manage the carbon footprint (*Li et al. 2013*). Furthermore, it makes ecological sense to utilize wastes from industries and convert them into useful resources such as energy (*Yuan et al. 2006, Dewulf and Van Langenhove 2005*).

An MFC is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms (*Allen and Bennetto 1993*). Research into MFCs has already been done on certain electrochemically active bacteria. Among these are the electrochemically active bacteria, Shewanella putrefaciens (*Kim et al. 1999*) and *Aeromonas hydrophila* (*Pham et al. 2003*).

Microbial isolates can produce hydrogen, methane and/or methanol or electricity directly and transfer their electron production (*Logan 2008*). Research is being conducted to determine the optimum substrates (wastes), microbial mix, anode and cathode electrode construction, and parameters (pH, DO, temperature). This is to obtain the highest energy output at reasonable cost (*Zhang et al. 2011*).

Uses of MFCs are varied and expanding. For example, MFCs were used for waste treatment (*Aelterman et al. 2006, Shizas and Bagley 2004*), bioremediation (*Reimers et al. 2001*) and hydrogen production (Liu et al. 2005). Other uses include robotics (*Ieropoulos et al. 2003, Santoro et al. 2017*), recovery of phosphate (*Ichihashi and Hirooka 2012, Cusick and Logan 2012*) and recovery of nitrogen (*Kuntke et al. 2012*).

In terms of substrates, a review of synthetic media was summarized by *Pant et al.* (2010). Domestic sewage was the only substrate that was complex in composition.

In this study, waste such as fish sediments are the most active substrates, using a putative isolate of *Enterebacter* species with ammonium sulphate increase activity and the performance of the MFC connected in series and in parallel is best in series.

MATERIALS AND METHODS

Soil, sediment, water, and corn stover were collected in sterile 1500 ml polypropylene tubes and stored in a dark cooler. An improvised MFC was constructed using PVC pipes and food jars (*Hotingoy 2010*). The microbial fuel cells were constructed using 800 mL plastic containers that were paired up and joined by a ¾-inch diameter by 2-inch long PVC pipe using epoxy clay (VulcaSeal, Philippines). The pipes were then filled with a standard mixture of agar (Scharlau, Spain) (15g/L) and table salt (sodium chloride) (150g/L) that served as salt bridge. Each sample was covered with 1 cm thick oil film (Minola, Philippines) poured on top of the sample to ensure an anaerobic condition for bacterial growth. Graphite lead from commercially available pencils (Victory, Philippines) was used as electrodes in both the anode and the cathode chambers. Copper wires were used to attach the electrodes together with a 1.0 Ω resistor in between. The cathode chamber was sparged by air using a 220 v standard aquarium pump (Precision, China).

The two compartments of the MFC device consisted of the anode and cathode sections separated by an agar plug (15% w/v). Each compartment contained 750 ml capacity (**Figure 1**). The resistor had a resistance of 1 Ω.

Total volumes of substrates were kept constant at 750 ml, concentrations of substrates were varied. Temperature was kept at 25°C. Several treatments were used. Domestic sewage was obtained from an outflow pipe originating from the University of the Philippines Visayas, Miag-ao Campus, Iloilo, Philippines (10.640198 N, 122.231460 E). Paddy water was obtained from a rice field on the roadside towards Miag-ao, Iloilo, Philippines (10.642760 N, 122.25217 E). Seawater was obtained from the beachfront of UP Visayas, Miag-ao Campus, Iloilo, Philippines. Sediment was obtained from the UP Visayas milkfish fishpens at Leganes, Iloilo City, Philippines while corn stover as obtained from a farm in San Juan, Antique, Philippines. Fish feed (Interfeeds Inc., Philippines) was obtained from a commercial store in Iloilo City. Ammonium sulfate, technical grade was obtained from a local chemical supplier (Valiant Chemical Inc., Philippines). A pure culture of a putative *Enterobacter* species was isolated from the sewage sample. MacConkey agar plus 0.5% glucose was used as differential media (*Elazhary et al. 1973, Bruce et al. 1981*). To inoculate the *Enterobacter* species (EC), the culture was incubated overnight in 100 ml nutrient broth (Hi Media, India). At approximately 5 x 10 6 mL^{-1} (O.D. = 0.6), of the culture was then added to an MFC at 10% v/v, as required. Each treatment was done in triplicates. Combinations of substrates for each treatment were described. For paddy water, domestic sewage, and seawater, treatments consisted of pure paddy water, domestic sewage or seawater. Sediment treatments consisted of 30%, 40%, and 50% w/v sediment. Additional sediment treatments consisted of addition of 10%w/v fish feed (feed) and/or 7 % w/v ammonium sulfate (AS). For corn stover treatments, the treatments consisted of corn stover at 15%, 25%, and 35% w/v. Additional treatments for corn stover experiments included addition of 7% (w/v) of ammonium sulfate and addition of 10% (v/v) of *Enterobacter* sp. culture.

Control MFC consisted of both anode and cathode cells containing sterile water only.

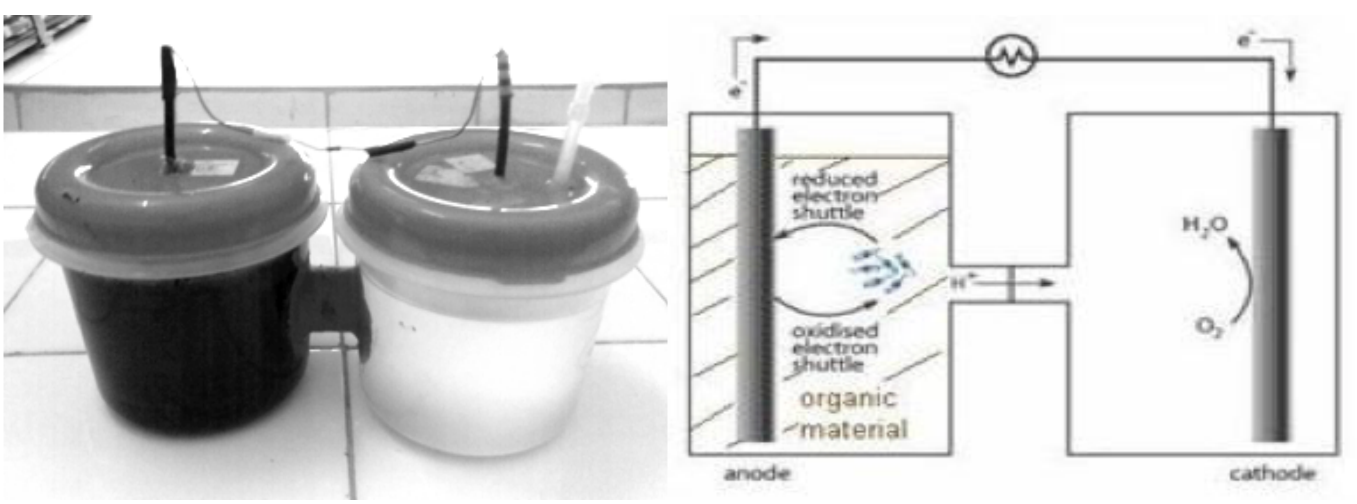

Figure 1. Improvsied MFC using PVC pipes (A) and working principle (B).

Measurements

Voltage, amperage, and columbic efficiency were determined using a multimeter (Broadway Multimeter, Japan). Measurements were taken daily after set up. Since MFCs are designed to maximize total system power, ultimately the most important factor is the power production on the basis of the total reactor volume. This was calculated by:

$$P_v = E^2_{MFC} \ / \ vR_{ext}$$

where P_v, is the volumetric power (mW/m^3); E^2_{MFC} is the measured voltage (V); v the total reactor volume (ml) and R_{ext} (Ω) as the external resistor (*Logan 2008*).

Series-parallel analysis and charging feasibility analysis

To determine the effects of MFCs in series or in parallel, individual MFC cells were connected in series and parallel combinations to determine voltage and amperage changes (**Figure 2**).

Statistical analysis

Statistical analyses were performed on each treatment using ANOVA and DMRT *(Gomez and Gomez 1984*).

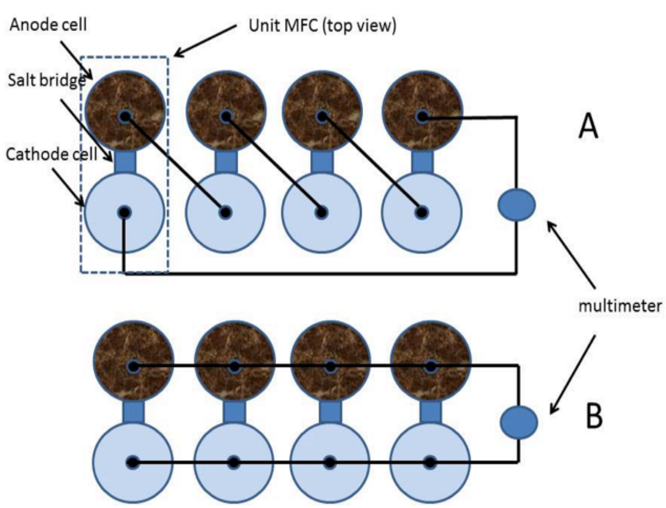

Figure 2. Improvised MFC combined in series (A) and parallel (B).

RESULTS AND DISCUSSION

Fish pen sediment treatments produced the highest voltage across treatments. Although 40% sediment produced the highest voltage (766 mV) 30% sediment treatment showed consistently high values. Treatment with 40% appeared to be a limit as 50% sediment showed less voltage output then 40% or 30% sediment. Treatments with the addition of feed and addition of feed and ammonium sulfate caused a decrease in the overall voltage output of sediment treatments. Addition of feed and ammonium sulfate also delayed the rise in voltage. Treatments with corn stover showed the opposite effect. Treatments with corn stover alone did not reach values above 150 mV. Treatments of corn stover with the addition of a putative isolate of *Enterobacter* sp. reached values less than 300 mV of addition and treatments with *Enterobacter* sp. and ammonia reached values less than 550 mV. Treatments of domestic sewage, paddy water, and seawater did not produce voltages more than 75 mV. Un-appended treatments seemed to decrease in voltage at days 11 to 12. This was however due to a power outage that affected the air compressor supplying oxygen to the cathode (**Figure 3**).

For amperage, results in general were similar to the voltage pattern. Although the highest current output was shown by the treatment with 30% sediment + fish feed + ammonium sulfate (967 mA), treatments with 40% sediment and 30% sediment treatment consistently showed the highest daily values (950 mA and 860 mA, respectively). The treatment with 50% sediment only showed 550 mA as the highest value. The increase in current was also delayed by the addition of fish feed and ammonium sulfate. Sediment treatments appended by additives showed a 4-5 days lag compared to the un-appended treatments. Corn stover treatments did not produce current more than 100 mA. However corn stover treatments appended with *Enterobacter* sp. and ammonia seemed to increase current output 900% compared to the un-appended treatments (**Figure 4**).

For power density, the highest observed value was after 13 days using 30% sediment appended with fish feed and ammonium sulfate (186 mW.m^{-2}). Treatments with 40% sediment and 30% sediment treatment had the consistently higher daily values (180 mW.m^{-2} and 128 mW.m^{-2}, respectively). The treatment with 50% sediment only had a maximum power density of 64 mW.m^{-2}. The increase in power density was also delayed by the addition of fish feed and ammonium sulfate. Sediment treatments appended by additives showed a 4-5 days lag compared to the un-appended treatments. Corn stover, domestic sewage, paddy water, and seawater treatments did not produce power densities more than 0.5 mW.m^{-2} (**Figure 5**).

The effect of ammonia on sediment was unexpected

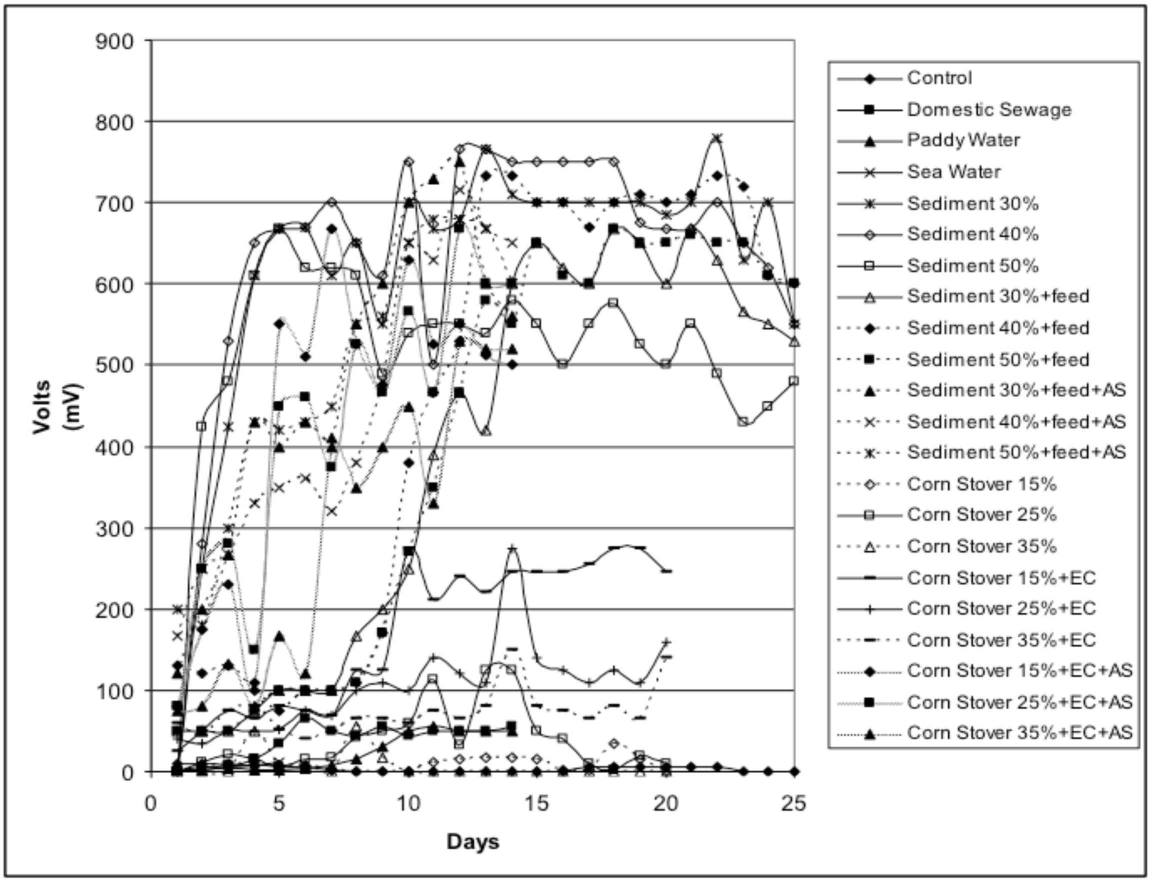

Figure 3. Comparison of voltage outputs among treatments.

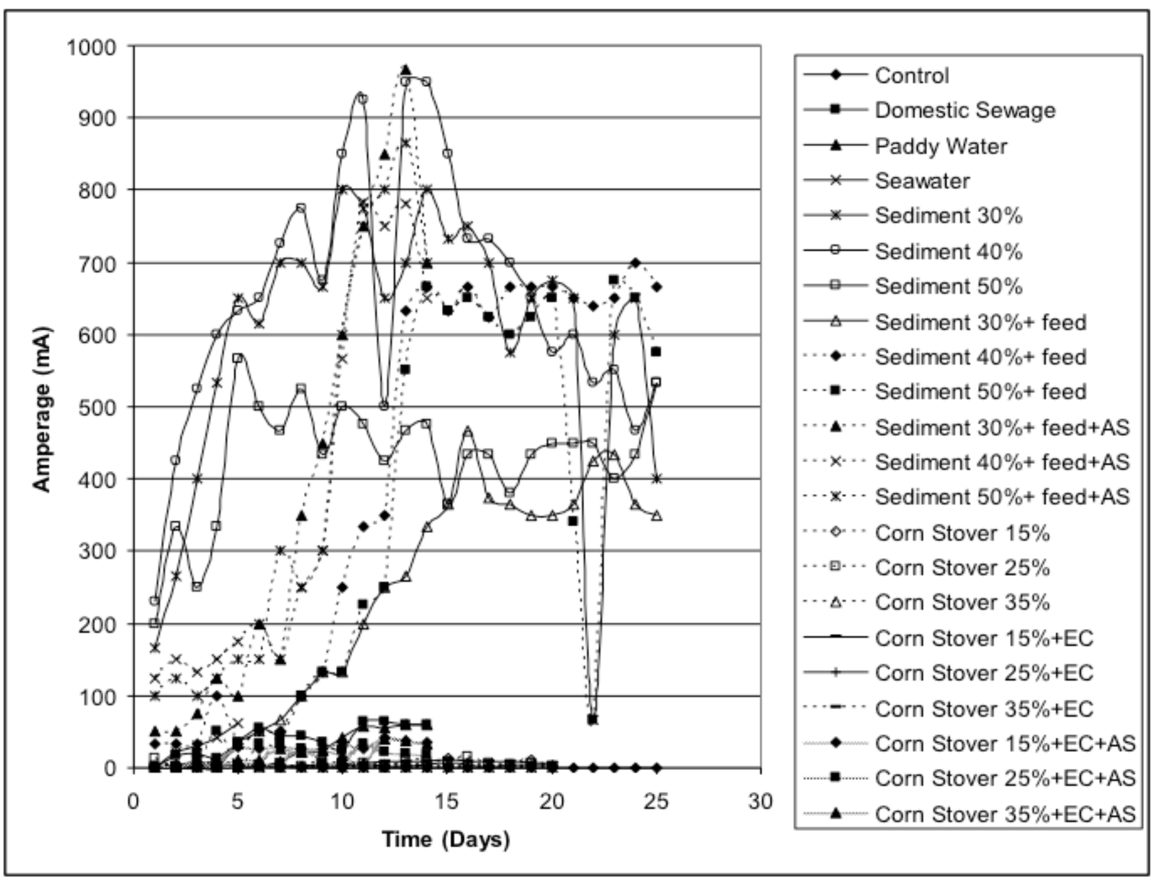

Figure 4. Comparison of current outputs among treatments.

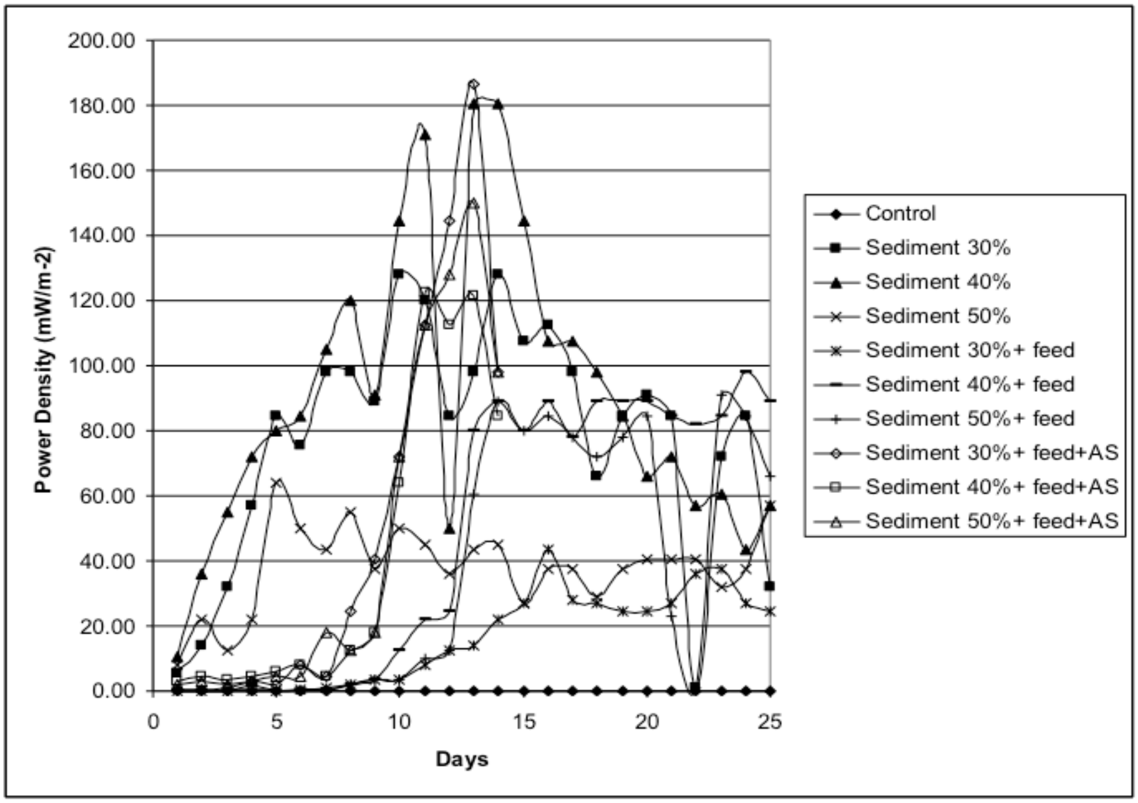

Figure 5. Comparison of power density outputs among treatments.

whereas the effect of ammonia on corn stover was similar to those found in previous experiments (H*e et al. 2009*). Apparently, the nitrogen cycle is not the pathway for electrogenesis. Fish feed, which is a complex nutrient source, may have inhibited electrogenesis. The two possible reasons are: the feed was mixed with antibiotic that inhibits bacterial growth; and the complex mix is not suitable for the type of electrogenic bacteria present in sediment. The complex nutrients in the feed might need to be broken down to simpler amino acids before it is utilized by the bacteria. This reason was reflected in the addition of ammonium sulfate. Ammonia, a source of nitrogen for bacteria, helped increase the electrogenic output but not as high as un-appended treatments. A possible group of microorganisms that may be responsible for electrogenesis are those belonging to the sulfur cycle (*Varma et al. 1983*).

Observations beyond 25 days are currently difficult. The physical integrity of the agar plug that separated the cathode and anode chamber broke down. Both chambers leaked contents into the others' space and resulted in a shorted circuit. The break down may be due to agarolytic bacteria degrading the plug (*Aoki et al 1990*).

In another trial, arrangement of four individual MFCs connected in series using 30% sediment improved the voltage up to 2.4 V and 0.009 A compared to any individual MFC (maximum 0.75 V and 0.005 A, respectively). This was followed by treatment using 30% sediment arranged in parallel and 10% sediment + feed arranged in series. Other remaining treatments did not give comparable results (**Figures 6 and 7**). Increase in voltage after connecting the MFCs in series was expected. It was not expected that the higher amperage was obtained in the series configuration than in the parallel configuration. Normally, a parallel configuration would produce higher amperage compared to a series configuration (*Resnick and Halliday 1966*). However the amperage in each treatment was not significantly different.

Power density of the 30% sediment MFC treatment arranged in series, therefore, had the highest power density (0.130 mW/m^3). It was significantly different from the 30% sediment MFC treatment arranged in parallel (0.025 mW/m^3) and other treatments (**Figure 8**).

With these results, wastes that could normally be discarded, underutilized, and generate greenhouse gases such as methane could be used as energy sources in unconventional ways (*Holmer and Kristensen 1994 and 1996, Li et al. 2013*).

This follows the industrial ecology principle wherein wastes of one industry are a resource for another (*Yuan et al. 2006, Dewulf and Van Langenhove 2005*).

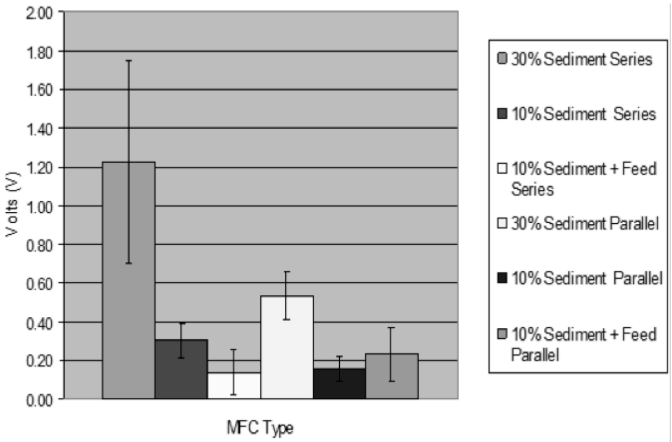

Figure 6. Comparison of voltage for each treatment in series and parallel Error bars in SD.DMRT values in small letter at 0.05% level of significance.

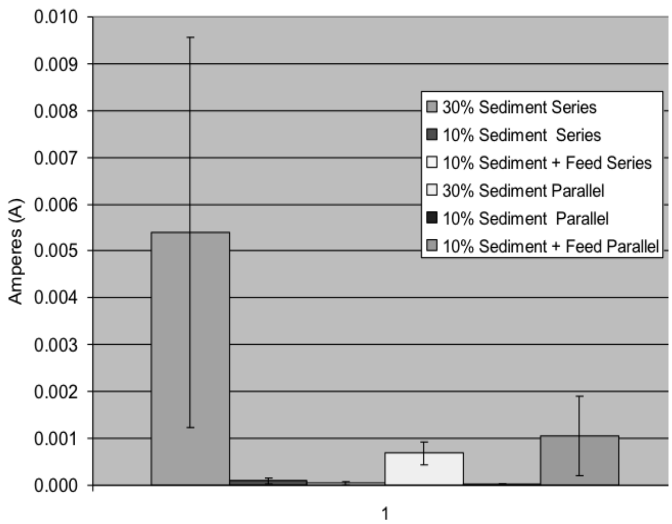

Figure 7. Comparison of amperage for each treatment in series (A) and parallel (B) Error bars in SD. Treatments were not significantly different at 0.5% level of significance.

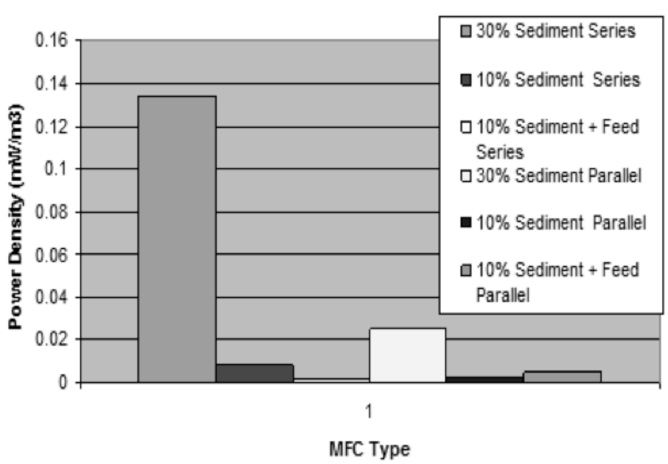

Figure 8. Comparison of power density for each treatment arranged in series and parallel configuration.

This ideal situation should be strived for to achieve sustainability in terms of energy resources.

CONCLUSION AND RECOMMENDATIONS

Microbial fuel cells can be made from wastes and substrates from fish farm sediment, corn stover, domestic sewage, and paddy water. However, the most practical MFC can be made from 30% and 40% fish farm sediment. Addition of ammonia decreased the power density of the fish farm sediment MFC implying another electrogenic pathway (most likely sulfur based). Ammonia, however, increased the power density of corn stover MFCs. In addition, arrangement of the fish sediment MFC in series increased voltage and current suitable for charging 1.5V batteries. Further studies are required to look at other local wastes as substrates and the possibility of the sulfur-based electrogenic pathway. Analysis of the components of the fish farm sediments to determine the cause(s) and the main contributor to the electrogenic pathway is also needed.

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