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# A study on the electricity generation from the cow dung using microbial fuel cell

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## Abstract

Microbial Fuel Cells (MFCs) use bacteria as biocatalyst to convert biodegradable substrates into electricity. The natural cow dung was found more suitable as it generated 150.9 mV Open Circuit Voltage (OCV) against 3.2 mV OCV generated by the sterile cow dung in the H-shaped MFC. On configuration, a MFC with 50 % cow dung and a salt bridge (5 cm × 2 cm) containing a mixture of 10 % sodium chloride and 5 % agar, electrodes of 32.20 $cm^2$ surface area and phenol red (0.1 %) as exogenous mediator was found ideal. Furthermore, five electrogenic bacteria were isolated from the cow dung and individually studied for their electrogenic properties. The bacterial isolate CD64 was found best among the isolates, as it generated 710.7 mV OCV at 37 ºC, pH 7.0 with LB medium as anolyte against vinegar as catholyte. Based on the phenotypic characteristics and 16S rDNA sequencing, isolate CD64 was identified as *Bacillus* sp.


**Key words:** Bio-electricity, Cow dung, Electrogenic bacteria, Microbial Fuel Cell, Open Circuit Voltage, Salt bridge

## Introduction

The world today is undoubtedly facing a serious energy crisis and energy demand continues to increase at an unsustainable pace. New methods of electricity generation from renewable resources without a net carbon dioxide emission are much desired (Lovely 2006). Recently, MFC have drawn increasing world-wide attention in generating electricity directly from organic matter (Kim et al. 1999; Chaudhuri and Lovely 2003; Rabaey et al. 2003; Liu and Hogan 2004; Min and Logan 2004). The MFC can be considered as bioreactor using bacteria as catalysts, which convert chemical energy stored in biomass to electricity (Kim et al. 2007; Frank et al. 2007). Typical MFC consists of an anaerobic anodic zone and an aerobic cathodic zone separated by a proton exchange membrane (PEM). Bacteria attached at the surface of the anode act as biocatalysts to pull electrons out from substrates (Bond and lovely 2003; Rabaey et al. 2004).

Bacteria which are useful in the MFCs operation have the ability to transfer electrons to an electrode (anode), as terminal electron acceptors are classified as Electrochemically Active Bacteria (EAB) or Electrogenic bacteria (Rabaey et al. 2004). Electricity generation using domestic wastewater, swine wastewater, landfill leachate and corn stover hydrolysates as fuel were reported using several MFC configurations (Liu and Hogan 2004; Min and Logan 2004; Min et al. 2005; You et al. 2006; Zuo et al. 2006). The MFCs have also been reported to utilize a variety of individual substrates found in dairy manure such as acetate, cysteine, proteins as well as lignocellulose (Liu et al. 2005; Logan et al. 2005; Heilmann and Logan, 2006; Rismani et al. 2006). Agricultural manures from animal confinements can be ideal candidates as bioenergy feed stocks because of their high organic matter content, which is easily degradable. This manure has long been valued as a fertilizer, but it also leads to significant air and water pollution. The MFCs can be another option to gain value from the livestock manures through electricity generation and also reduce its pollution and concurrently maintain fertilizer value.

Most of the research performed on MFCs is concerned with increasing its power density but a very little work has been done on determining the bioelectricity generation from the MFC using cow dung as fuel and isolating potential electrogenic bacteria present in it. The present study explores the usage of cow dung as anolyte against sewage water as catholyte and establishes the possibility of alternate source of electricity by MFC using such wastes.

## Materials and Methods

*Collection of samples*

The cow dung samples of the breed Zebu Cattle (*Bos indicus*) were collected in sterile containers along with a liter of sewage

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wastewater from Meerut, Uttar Pradesh, India and stored at 4 ºC. Initially, the studies were carried with cow dung, as a sole anolyte against vinegar, to note the bioelectrical contribution of the microbial flora present in the cow dung.

*MFC fabrication and operation*

The H-shaped MFCs were fabricated with two polycarbonate bottles (500 mL) as chambers and a PVC pipe (5 cm × 1 cm) for preparing a salt bridge. The slat bridges were prepared by filling boiled sodium chloride (10 %) solution containing 5 % agar. The salt bridges were fixed to the bottles with the aid of epoxy adhesive (M-Seal, Pidilite Industries Ltd, Maharashtra, India).

The cow dung was used as anolyte without any pretreatment and vinegar was used as a catholyte in the MFC setups. The two graphite pencils (0.25 cm × 10 cm, Apollo pencil manufacturers, Mumbai, India) with surface area of 16.10 $cm^2$ were used as electrodes. To aid initial wetting, the electrodes were boiled in deionized water and soaked overnight in 1M HCl solution followed by thorough rinsing with deionized water (Allen and Bennetto, 1993).

The electrodes were inserted into respective chambers while circuit connections were set with the copper wires fixed into the drilled holes of the electrodes and sealed with epoxy resin to avoid corrosion of copper wire (Kim et al. 2002; Zou et al. 2007). The fabricated MFCs were sterilized with Ethanol (70 %) and irradiated with UV for 15 min. The electrolytes were added up to the brim of the respective chambers to maintained air free conditions.

Following 24 h incubation/inoculation, OCV from the MFCs was recorded at room temperature without any external resistance until 40 min with an interval of 2 min using the multidigital meter (UNI-DT830D, Uni-Trend Group Ltd., Kowloon, Hong Kong).

*Optimization of MFC parameters*

Since, it is necessary that MFCs should be optimized in terms of reactor configuration, MFCs were operated to optimize the following parameters and potential difference was monitored in terms of OCV.

*Effect of Cow dung concentrations*

The MFCs were operated with different concentrations (100, 75, 50, and 25 %) of normal cow dung against 500 mL of vinegar as catholyte while setup having 100 % cow dung and distil water in the anode chamber was referred as control.

*Effect of different salts and concentrations of ideal salt*

Ten percent sodium chloride, potassium chloride, ammonium nitrate, ammonium chloride and mixed salts (2.5 % each) were tested for the efficient conductivity of ions in the salt bridge. The salt which could produce higher OCV was further analyzed at different concentrations (5, 15 and 20 %).

*Effect of salt bridge length and radius*

Different lengths of the salt bridge (1.5, 3 and 5 cm) filled with the optimized concentration of salt, were analyzed for their effect on the OCV. Similarly, keeping the optimized length into consideration, the radius of the salt bridge was varied (1 cm and 2 cm) and analyzed.

*Effect of anode surface area*

The electrodes with an apparent surface area of 16.10 $cm^2$ and 32.20 $cm^2$ were used to monitor the effect of electrode surface area on the potential difference generated in the MFCs.

*Effect of external mediators*

The MFCs amended with exogenous mediators such as phenol red and neutral red (0.1 %) in the anodic chambers were analyzed for their contribution in the OCV production.

*Combination of cow dung with sewage water*

The optimally fabricated MFC setup was monitored for the OCV generation with the normal cow dung as anolyte against 500 mL of sewage water as catholyte.

*Isolation of bacteria from cow dung*

The cow dung was serially diluted with 0.85 % NaCl and $10^{-5}$ dilution (0.1 mL) was spread on the LB agar plate (Tryptone 10.0 g, NaCl 10.0 g, Agar 20.0 g and Yeast extract 5.0 g in 1000 mL distilled water) further incubated for 24h at 37 ºC. Morphologically distinct colonies obtained on the plate were further purified and studied for their Gram staining properties (Smibert and Krieg, 1994).

*Optimization of physiochemical growth parameters*

The LB broth inoculated with 24 h old cow dung isolates culture (1 %, v/v) were studied for the bacterial growth at different pH (4, 5, 6, 7, 8, and 9) and temperature (27, 37, 47 and 57 ºC) while measuring their absorption at 660 nm, against the sterile LB broth as blank (Safia et al. 2007).

*Electricity generation and identification of the electrogenic bacteria*

Using optimized growth and MFC parameters, the isolates were studied for their electrogenicity with 500 mL LB broth culture (24 h old) as anolyte against 500 mL vinegar as catholyte. The potential isolate was then outsourced to Bioserve Biotechnologies Pvt. Ltd, Hyderabad, India, for molecular identification by 16S rDNA technique. The 16S rDNA sequence obtained was then initially analyzed at NCBI server (http://www.ncbi.nlm.nih.gov/) using BLAST (Blastn) tool and corresponding sequences downloaded were further used for the phylogenetic analyses using MEGA version 4 (Tamura et al. 2007).

## Results and Discussions

*Analysis of bioelectricity generation from cow dung*

A constant elevation in the OCV was observed with maximum OCV generation of 150.9 mV at 40 min by normal cow dung MFC (Figure 1) whereas the maximum OCV of 3.2 mV was noted with oscillations from the MFC containing sterile cow dung. The results obtained confirmed the presence of electrogenic bacteria in microbial flora of the normal cow dung. Hence, the cow dung can be used as fuel in the MFC while bacteria present in it could engage the anode in their metabolism, resulting in the bioelectricity generation.

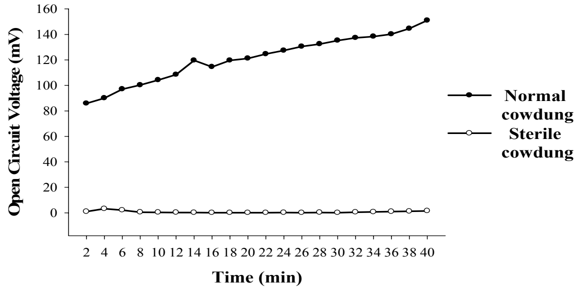


Figure 1: OCV from the normal cow dung and sterile cow dung over time

*Optimization of MFC operation*

*Concentration of cow dung*

Different concentrations of normal cow dung dilutions were further studied in the MFCs. The maximum OCV of 179.7 mV was recorded from MFC with 50 % cow dung at 40 min (Figure 2). It implies that at 50 % concentration of cow dung nutrients would be in sufficient amount easily available to the bacteria cause maximum OCV.

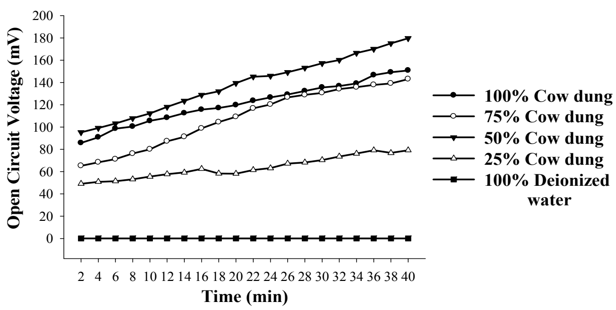


Figure 2: OCV generation from different concentrations of normal cow dung

*Analysis of different salts and optimized salt concentrations*

Among the different salts studied, the maximum OCV of 184.9 mV was recorded by the salt bridge containing NaCl (Figure 3) at 40 min with continuous and steady elevation in the electric potential over time in comparison to other salts. Hence, the NaCl was opted as the suitable salt which was further analyzed for its effective concentration for better OCV.

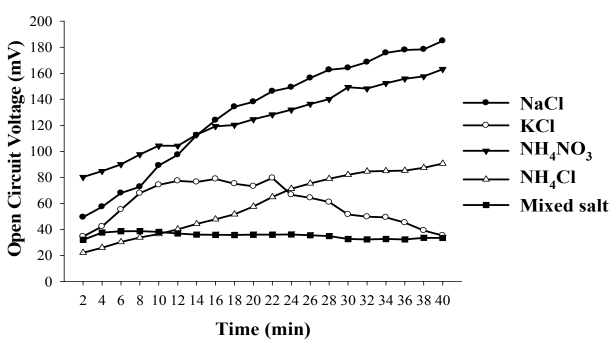


Figure 3: Bioelectricity production from the MFCs having salt bridges filled with various salts

The concentration of NaCl was further varied and observations revealed that 10 % could yield a maximum OCV of 199.5 mV at 40 min with consistent increment over time (Figure 4). This analysis recommended the use of 10 % NaCl concentration as optimized salt in the salt bridge to facilitate easy ion flux. It was concluded that the sodium chloride in the salt bridge yielded good OCV might be because of its good electrolytic property (Liu et al. 2005)

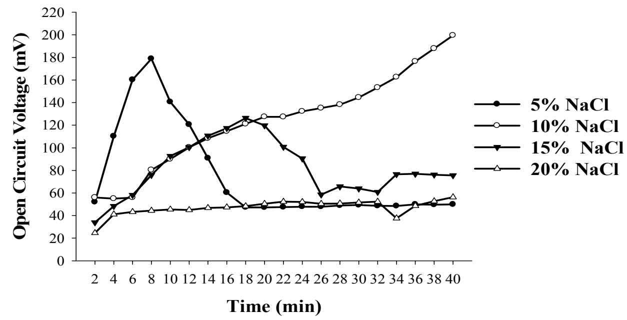


Figure 4: OCV from various concentration of salt used in the salt bridge

*Dimensions of salt bridge*

Based on the optimized NaCl concentration, various lengths of the salt bridge were monitored. The highest OCV generation of 203.6 mV from the salt bridge having 5 cm length with a uniform increment over time (Figure 5).

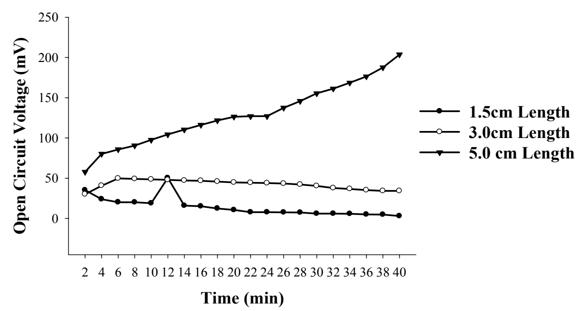


Figure 5: Monitoring of OVC from MFCs having different salt bridge length

The salt bridges having 5 cm length were varied in radius and tested. The OCV profile obtained reveals that the salt bridge with 2 cm radius could generate more OCV (199.6 mV) at 40 min compared to that of 1 cm radius (Figure 6). It can be explained on the basis that the 5 cm length and 2 cm radius of the salt bridge might have reduce the resistance of the salt bridge to the proton flux through it and hence resulted in maximum OCV production.

*Anode surface area*

Figure 7 represents the OCV from MFCs with the anode surface area of 16.10 $cm^2$ and 32.2 $cm^2$. The maximum OCV (212.9 mV) was recorded with 32.2 $cm^2$ anode surface area at 40 min. The results indicated that anode with 32.2 $cm^2$ surface area was ideal for the MFC configuration. It was concluded that maximum the anode surface area more would be the surface available to the bacteria to liberate electron that resulted in maximum OCV production.

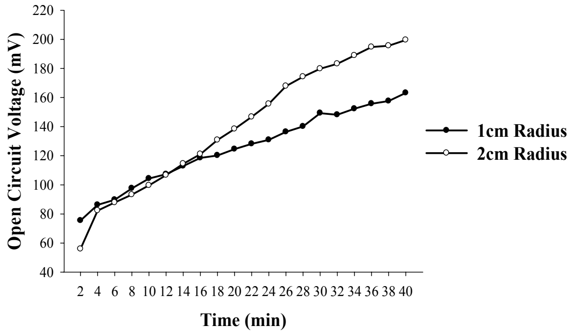


Figure 6: Bioelectricity generation from salt bridge with different radius

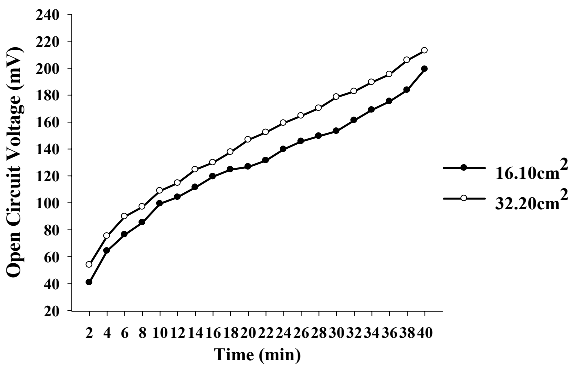


Figure 7: OCV recorded with the different surface area of anode

*Analysis of exogenous mediator*

The electricity generation in terms of OCV were recorded with phenol red and neutral red amended as electron shuttles in the anodic chamber containing 50 % cow dung. The maximum OCV of 245.5 mV for phenol red at 40 min better than that of neutral red (154.6 mV) was recorded(Fig.8). The data obtained concluded that the phenol red was more effective exogenous mediator than the neutral red.

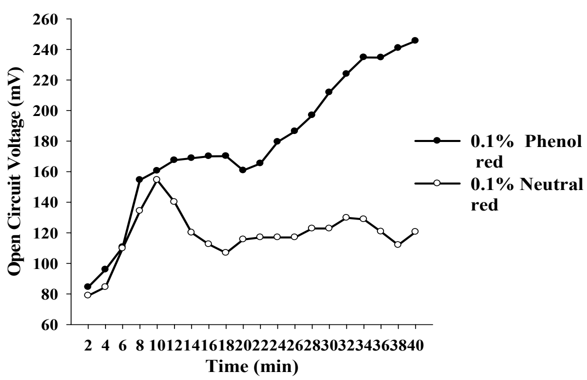


Figure 8: Electric potential monitored on addition of exogenous mediators.

The application of phenol red as exogenous mediator did show the significant increment in the voltage with respect to time while neutral red failed to do an increment in the OCV over time suggested the toxic effect of neutral red as most of the mediators are toxic for the microbes (Rabaey et al. 2003).

*Analysis of electricity generation from cow dung against sewage water*

The fabricated MFC setup was operated with all the optimized parameters studied and production of electricity was recorded after 24 h of inoculation in terms of OCV from MFC with 50 % cow dung as anolyte against 500 mL untreated sewage water as catholyte. The MFC with sewage water as catholyte could produce (Figure 9) maximum OCV of 195.6 mV at 40 min, which was better than the OCV (179.7 mV) of MFC containing vinegar as catholyte (Figure 2). Hence, results suggested that sewage water can be used as better catholyte.

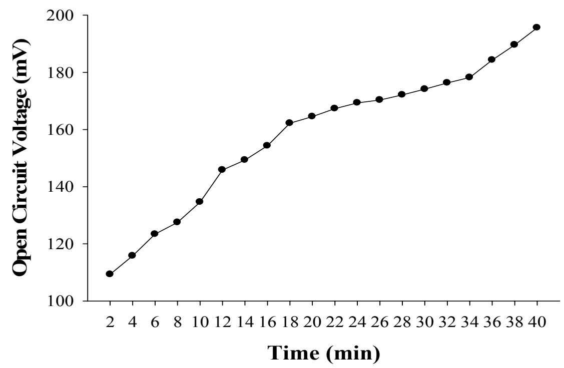


Figure 9: Production of OCV from MFC with sewage water as catholyte.

The sewage water as catholyte, performed better than the vinegar in the MFC. This indicates that sewage water can reduce the proton and electrons liberated from the anode chamber because of the presence of dissolved oxygen in it (Bond et al. 2002; Tender et al. 2002) which acts as electron acceptor having high thermodynamic redox potential.

*Study on cow dung bacterial isolates*

The five distinct bacterial colonies isolated were named as CD8, CD9, CD10, CD64 and CDS10. Gram staining study revealed that the cow dung isolate CD8 was gram-negative bacillus; CD9 was gram-positive cocci; CD10 was gram-negative bacillus; CD64 was gram-positive bacillus and CDS10 was gram-positive cocci in nature. The pH 7.0 and 37 ºC was the optimized requirements for the growth of all the bacterial isolates.

*Analysis of Bioelectricity generation from the cow dung isolates and identification of potential electrogenic bacteria*

All the five isolates were independently studied for their production of electricity generation using optimized pH7 and temperature 37 ºC. Figure 10 represents the OCV generation from the 24 h old bacterial isolates recorded for 40 min at the 2 min intervals, inoculated in the respective MFC chambers. It was recorded that the MFC containing isolate CD64 showed the maximum OCV of 710.7 mV at 40 min, with significant steady increase over time in comparison to rest of the isolates in the MFCs. These results deduced that isolate CD64 contributed more effectively into overall voltage generation from the normal cow dung where other strains were equally competitive except isolate CD10 which displayed the fall in OCV after a 14 min during MFC operation.

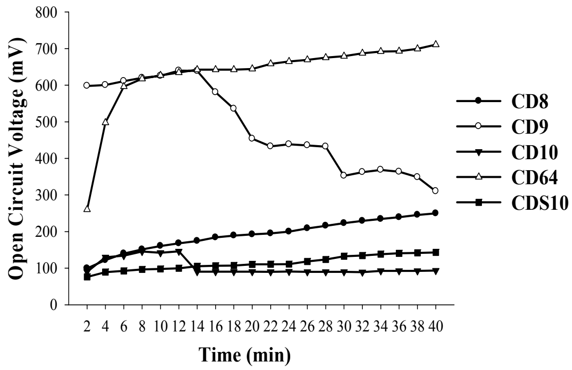


Figure 10: Generation of OCV profile of bacterial isolates from cow dung

Furthermore, the 16S rDNA sequence analysis of the bacterial isolate CD64 showed 99 % similarity with the species *Bacillus* sp. SRC_DSF22 (GU797304). Based on the phenotypic characteristics

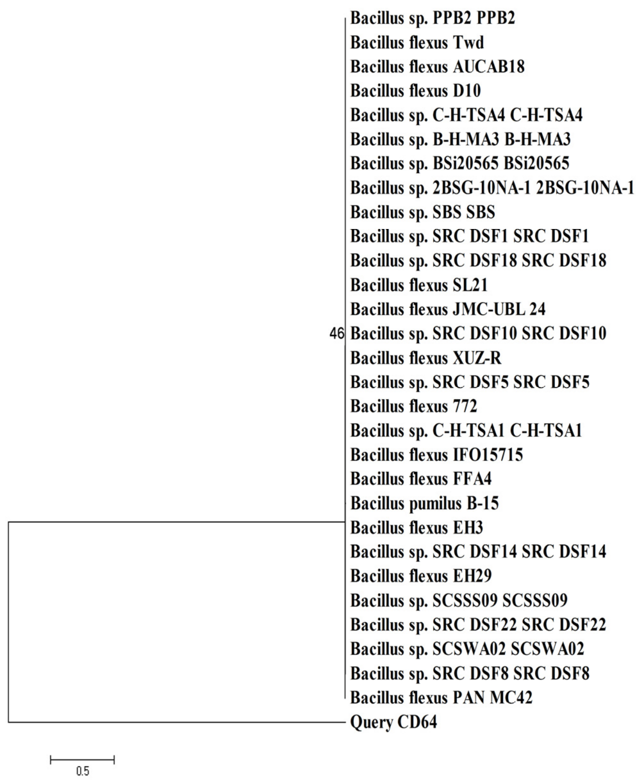


Figure 11: Phylogenetic tree of the isolate CD64 in relation to *Bacillus* sp. The tree was constructed using the neighbor-joining algorithm. Numbers on the tree refer to bootstrap values on 1000 replicates. The bar indicates a 0.5% estimated difference in nucleotide sequences

and phylogenetic analysis using 16S rDNA, isolate CD64 was identified as *Bacillus* sp. Figure 11 shows the phylogenetic relationship between the isolate CD64 with the *Bacillus* sp. The 16S rDNA sequences for the isolate CD64 have been deposited at Gene Bank in NCBI with accession number JQ814756.

It was already documented that MFC voltages will never exceed a theoretical OCV of 1.14 V (Madigan, 2000). In the present study, the OCV of 701 mV was generated by the isolate *Bacillus* sp. The optimum pH noted for all the cow dung isolates were in accordance with the report in which highest bioelectricity generation observed thus far at pH 7.0 (Gil et al. 2003). The MFCs operational temperature of 37 °C for cow dung isolates is also advantageous as the decreased temperature from 32 to 20 °C, reduces the power output (Liu et al. 2005). CD64 nearer to that of maximum OCV of 800 mV (Liu, 2005) reported until now.

The present study also indicates that cow dung bacteria might have electrogenic properties like C-type cytochromes (Kim et al. 2002) or conductive nano-wires (Gorby et al. 2006) on their cell membranes to generate profuse bioelectricity. Such bacteria from cow dung can be used for bioelectricity generation in the MFCs like other electrogenic bacteria *Shewanella putrefaciens* (Kim et al. 1999) and *Geobacter sulfurreducens* (Kim et al. 2007).

## Conclusion

The present study establishes the usage of cow dung as the organic fuel and its bacteria as biocatalyst in the anode chamber along with sewage water as catholyte for the production of bioelectricity in the fabricated MFCs. The study also recommends the further tuning of the technology for the better usage of the waste towards the commercial utilization for the generation of alternate energy to sustain the demand of future.

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