

Isolation and Identification of Bacteria's from Cattle Dung used in Microbial Fuel Cells to Generate Bioelectricity

Gagandeep Kaur, I.K.Gujral Punjab Technical University, Jalandhar, Punjab, India

Yadwinder.S.Brar, I.K.Gujral Punjab Technical University, Jalandhar, Punjab, India

D.P.Kothari, J.D.College of Engineering & Technology, Nagpur, India

Corresponding Email Id: simicgagan@gmail.com

Abstract: *In recent times, recycling of biomass based organic waste has emerged as very vital aspect of wastes management. Cattle dung is extensively studied for its use as bio agriculture fertilizers and widely explored as prominent, potential and alternative fuel. The unique characteristics to carry diversified microbes in dung are exploited to use it as substrate in recently developed novel technology of Microbial Fuel Cells (MFC). In this study an inexpensive lab scale H-shaped double chamber MFC consisting of two chambers separated by salt bridge was fabricated. Role of cattle dung for biological utilization based bacteria to make biofilm and generate energy in microbial fuel cell was observed. The research studies are able to enumerate Total Viable Count in the range of 1.9×10^6 to 2.8×10^6 cfu/gram of dung sample and subsequently primarily nine isolates *Bacillus subtilis*, *Escherichia coli*, *Streptococcus spp*, *Pseudomonas aeruginosa*, *Clostridium Spp*, *Peptostreptococcus Spp*, *Bacillus Cereus*, *Klebsiella Spp*, *Bacteroides Species*. While examining bio film majority of bacteria were of anaerobic nature which contribute substantially in bioelectricity generation. Undoubtedly, the exploitation of cattle dung bacterial community may contribute substantially in sustainability of energy generation in*

MFC, but to identify electrifying bacteria and thoroughly understanding the bio mechanism with the help of recent advances in research in engineering and biotechnology is a key of success.

Keywords: bacteria, biomass, biofilm, bioelectricity cattle dung, MFC

1. Introduction: Energy consumption across the globe has increased exponentially in recent past and to meet the ever increasing energy demand, there is a dire need to identify more and all feasible sources of energy (**Gagandeep: 2017**). Rigorous use of fossil fuels to meet demand has posed a threat to life with secondary effects of global warming and environmental pollution (**Lovely:2006, Rahimnejad: 2015**). In recent times, recycling of biomass based organic waste has emerged as very vital aspect of wastes management. Many of such wastes like municipal solid waste, food wastes, night soils and livestock manures such as cattle dung, sheep, goat dung, poultry drooping and plant wastes that can be very easily managed through biological methods. Cattle dung is the organic biomass mixture of semi-digested and undigested residues of digested matter excreted through the cow's digestive system from bovine's animal species. Cattle dung is extensively studied for its use as bio agriculture fertilizers and widely explored as prominent, potential and alternative fuel in the form of biogas with high methane values. Cow dung carries a wide diversity of microorganism containing different species of bacteria like *Bacillus spp.*, *Corynebacterium spp.* and *Lactobacillus spp.*, *Citrobacter*, *Enterobacter*, *Escherichia coli*, *Klebsiella spp.*, *Pasteurella spp* along with protozoa and yeast (**Nene: 1999, Kartikey: 2016**). Versatility of microorganisms in dung has encouraged the researchers to seek alternative sources for energy by utilizing traditional biomass sources by employing modern tools of technologies developed recently. As a consequence of these efforts, one of the recently proposed alternatives is energy derived from fuel cells utilising presently existing biomasses. Microbial Fuel Cells (MFC) are novel devices that use bacterial community as the catalyst for the oxidation of organic or inorganic matter and lead to generate current (**Logan: 2006**). Therefore a bioelectricity is generated in MFC between bacterial metabolic due to

development of biopotential (**Heilmann: 2006**). A bio-potential developed between the bacterial metabolic and these conditions leads to generate a bioelectricity in MFCs. Anaerobic conditions are necessary in anode chamber as oxygen will hinder the production of electricity (**Davis: 1962, Rahimnejad: 2015**). In present lab scale studies, an inexpensive and widely used configuration of lab scale H-shaped double chamber MFC consisting of two chambers separated by salt bridge have been assembled as shown in Figure 1. These H-shaped are widely acceptable for examining power production with different materials, microbes and mediators.

Research on role of cattle dung as a source of renewable energy subjected to optimum biological utilization based bacteria to make biofilm and generate energy in microbial fuel cell is on full swing. Our objective for this study is to isolate, identify and characterized the bacteria from cattle dung substrate with different morphological and biochemical basis and to study their usefulness in the MFC to make bio-film and subsequently generate electricity.

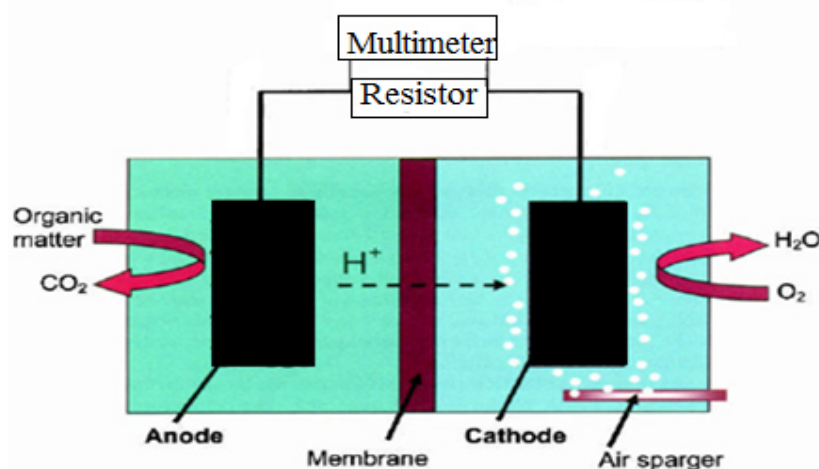


Figure 1: Schematic of Basic Components of Double Chamber MFC

2. Material and Methods: Cattle dung is naturally occurring rich organic matter having different bacterial colonies. In the present study, the random samples of recently excreted fresh cattle dung samples were collected from three different locations. All animals were routinely fed with locally available green fodder along with the concentrate ration.

Cattle dung is the main substrate used in the MFC along with the sterile distilled water or other medium. In MFC anodic chamber under anaerobic environment with some of the specific group of bacteria may increase in number and make a bio film on anode electrode and enhance power generating capabilities of cells by many folds. Therefore, enumeration of the bacteria in cattle dung substrate before being utilized in MFC, and then after removing bio-film from anode electrode.

2.1 Enumeration of Bacterial Population in Samples: Enumeration of bacterial population was determined by the serial dilution methods which is developed by (Frazier:1995, Talaro: 2009). For the microbiological analysis of dung, fresh cattle dung samples are homogenized thoroughly. For the preparation of samples, one gram of fresh dung, out of the collected dung is taken and mixed with 100 ml distilled water in beaker. The homogenized dung sample is marked as 1A and serially diluted in seven tubes with distilled water up to 10^{-7} . Further step, 0.1 ml of solution is taken from tube 1 and dropped on Petri plate already having the Nutrient Agar for growth of bacteria. Later the solution is made to spread on Petri plate and plate is ready to place in aerobic/ anaerobic conditions and so on for other. After incubation, the plates are analysed under microscope and colonies were counted with multiplier 10^1 . Similarly plate 2, had been analyzed and counted colonies were reported with multiplier 10^2 & so on. After that, colony forming units (cfu) are counted as total viable count TVC. Standard plate count method is used for culturing as well as calculating total viable count (TVC). The same serial dilution process is repeated for sample 1 B. The average of two TVC is taken as result. Again fresh cattle dung is collected from another two regions and marked it as sample 2 and sample 3 and same process of TVC determination is applied.

2.2 Isolation of Bacterial Colonies from the Cow Dung and Electrode Biofilm: During incubation, variety of colonies observed on the cultures plates are selected and purified with the help of selective media like brain heart infusion, eosin methylene blue agar, brilliant green agar and Macconkey lactose agar (MLA) . The different bacterial species isolated from fresh dung are determined by standard bacteriological identification methods according to Bergey's manual (**7th edition**). Morphological features like gram staining, shape and motility are examined. By using broth culture, various biochemical test are conducted which mainly includes Catalyze, Oxidase, Triple Sugar Iron, Sugar fermentation, IMViC series which consist of four definite tests i.e Indole production, methyl red, Voges Proskauer and Citrate utilization (**Talaro: 2009**).

3. Results and Discussion: Cattle dung is a special type of organic matter which is quite rich with bacteria. Under specific anaerobic conditions these bacteria grow in millions and help to act as active catalyst to increase the pace of chemical reactions. These reactions improve the power generation capability of cells. The conditions for enough microbial growth are maintenance of anaerobic environment, sufficient substrate and required pH value. The microbiological analysis of dung samples mainly includes total bacterial count which is necessary in MFC operation for transfer of electrons to electrode. Bacteria attach to the surface of the anode act as biocatalysts to pull electrons from substrate. The results of total viable count of bacteria, isolation and identification of bacteria are discussed below.

3.1 Total Viable Count of Bacteria: Total viable count (TVC) is an important parameter to evaluate the quality of dung to be used as manure as well as bio-energy source. Enumeration of microbial loads has been calculated as colony forming units (cfu/gram) of all the 3 dung samples. Each sample has been serially diluted in duplicate to calculate TVC and results are shown in table 1. Result shows that, average values of TVC are 2.8×10^6 cfu/gram, 1.9×10^6 cfu/gram and 2.5×10^7 cfu/gram in sample one, two and three respectively. From these

plated 15 distinct colonies were further streaked onto the general as well as selective culture medium to get the pure and isolated colony (Teo : 2011).

Table 1: Total Bacterial Count

Biomass	TVC A (cfu/gm)	TVC B (cfu/gm)	Average TVC (cfu/gm)
Cattle Dung Sample 1	3.4×10^6	2.2×10^6	2.8×10^6
Cattle Dung Sample 2	1.5×10^6	2.3×10^6	1.9×10^6
Cattle Dung Sample 3	2.9×10^7	2.1×10^7	2.5×10^7

3.2 Isolation and Identification of Isolates: Bacterial isolates (from the cattle dung as well as from the biofilm of anodic chamber) are classified on the basis of their features in different forms of species. To enhance the power generating capabilities of MFC, identification of power developing bacteria is significant. Identification has helped in addition of particular type of bacteria as mediator for augment of power density.

3.2.1 Morphological Features of Bacterial Isolates: In the present study, 15 bacterial species have been isolated from the dung samples. Only 9 (B1-B9) isolates have been selected for study and remaining six isolates are ignored being similar in morphological features. The detailed morphological characteristics of nine isolates mentioned in table 2 shows the distinct morphological features of bacterial isolates. The features include bacterial colony characteristics of colony shape, colour, size, margin, appearance, colour pigmentations, gram staining reactions, bacterial morphology, spore staining and motility. Some isolated bacteria colonies and their microscopic views of gram staining are shown in

figure 2. The results of bacterial colony characteristics of this study are comparable to (Dhadse: 2012, and Yuti : 2013).

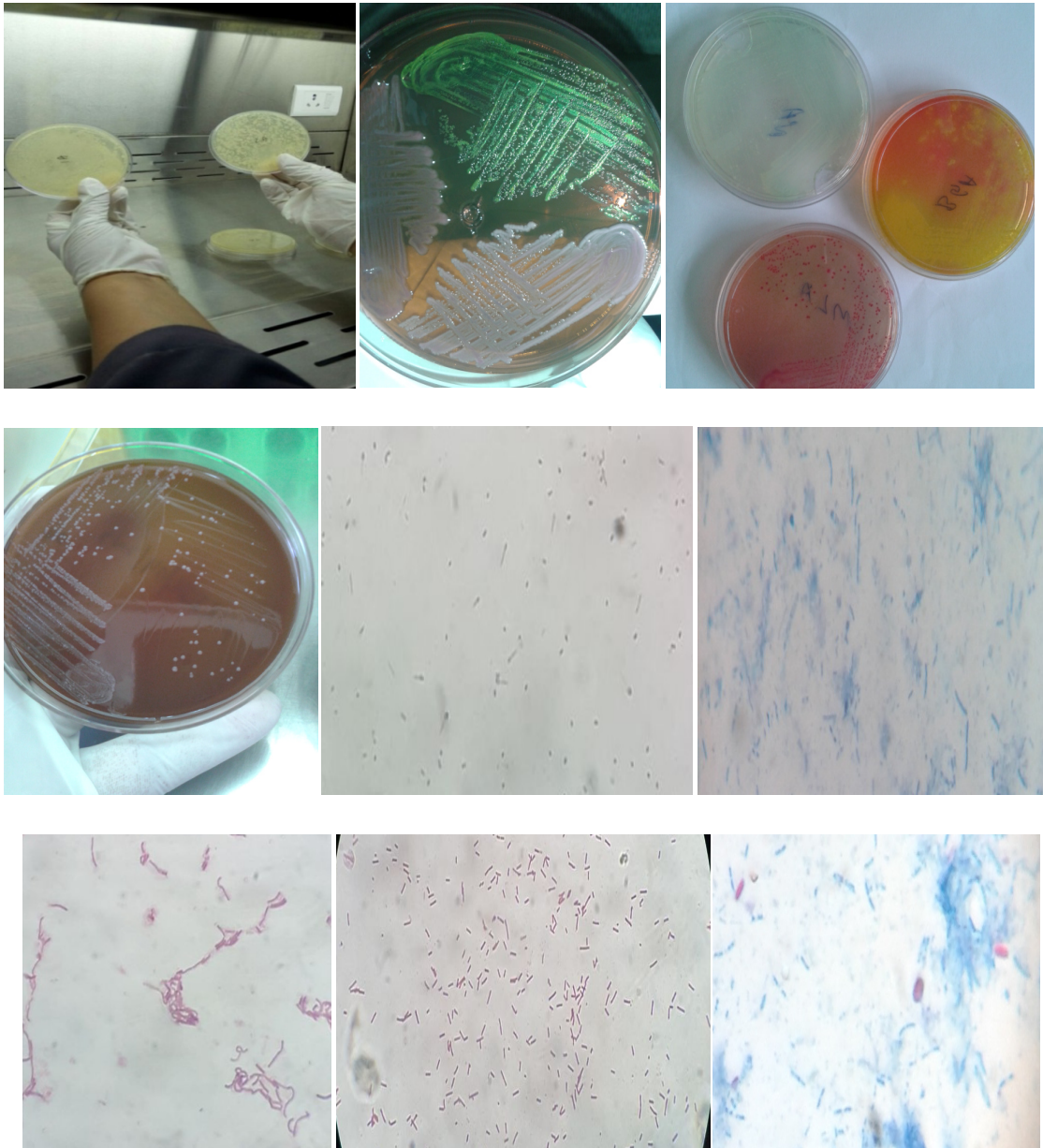


Figure 2: Isolated Bacterial Colonies and their Microscopic view with Gram Staining

3.2.2 Biochemical Analysis of Isolates: Nine bacterial species have been isolated from the cattle dung, characterised and identified by standard bacteriological identification procedure. Various kind of biochemical tests have been conducted by inoculating broth culture of the isolates into the variety of media and chemicals. These tests include variety of biochemical, enzymatic and Sugar Fermentation Test (Glucose, sucrose, maltose lactose etc.). Table 3 presents the biochemical and enzymatic features of bacterial isolates which includes biochemical tests, O-F test, methyl red test and citrate utilization test. Table 4 presents the sugar fermentation results of bacterial isolates which includes identifying the presence of mainly glucose, fructose, sucrose and lactose. Biochemical characteristics of isolated bacteria revealed extreme variations in their metabolic capacities. These unique features have been used to analyse and identify the isolates. The isolates are B1 (*Bacillus subtilis*), B2 (*Escherichia coli*), B3 (*Streptococcus spp*), B4 (*Pseudomonas aeruginosa*), B5 (*Clostridium Spp*), B6 (*Peptostreptococcus Species*), B7 (*Bacillus Cereus*), B8 (*Klebsiella Spp*), B9 (*Bacteroides Species*). On the anodic chamber biofilm contains almost anaerobic bacteria which could be the helpful in the generating electricity in the MFC. The major anaerobic bacteria B1 (*Bacillus subtilis*), B5 (*Clostridium Spp*), B6 (*Peptostreptococcus Species*), B7 (*Bacillus Cereus*), B9 (*Bacteroides Species*). The isolate findings of this study are comparable to Adegunloye: 2007, Gopinath: 2014, Shivkumar: 2012, Nene:1999, Sawant: 2007, and Kartikey: 2016.

4. Conclusion: The study suggests cattle dung is house of numerous varieties of microbes with unique and variable properties. Exploitation of cattle dung bacterial community may contribute substantially in sustainability of energy generation with agriculture waste. Undoubtedly, more detailed studies of micro flora of dung as a substrate and elaborated research on bio film components in MFC will prove to be a milestone. These will be positive steps to combat energy crisis and depleted fossil energy resources along with environmental problems that eventually controls the global warming. The optimum use of cattle dung micro flora with considerable potential can result in the eco-friendly and better sustainability. To identify electrifying anaerobic bacteria and thoroughly understanding the bio mechanism with

the help of effective utilization of recent advances in engineering and biotechnology is the need of an hour. There is tremendous scope for research and development in dung based MFC to take into the industrial field along with commercialization. On positive node it can precisely say that dung based biomass may be presumed to be an easily available bio-resource material that carries a huge potential to meet sustainable energy development in the near future.

Table 2: Distinct Morphological Features of Bacterial Isolates

Colony Characteristics	Isolated bacterial colony								
	B1	B2	B3	B4	B5	B6	B7	B8	B9
Colony shape	Rough/ Wrinkled	Round	Round	Round	Round	Round	Round	Round	Round
Elevation / Colour	Flat	Flat	Raised	Flat	Flat	Flat	Convex	flat	convex
Optical property / Size of colony	Opaque Small	Opaque Large	Translucent Large	Opaque Large	Dusky Large	Translucent Large	Cloudy Small	Opaque Small	opaque Small
Margin	Entire	Entire	Curled	Scattered edges	Undulate	Entire	Entire	Entire	Entire
Appearance	Dull & Mucoid	smooth	Mucoid	Shiny	Dull	Shiny	Shiny	mucoid	Moist

Colony colour	brownish Whitsh	Greyish White	White	Diffuse greenish	White	Blackish Yellow	Greenish White	Off White	Light Grey
Gram staining Reaction	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Negative	Negative
Bacterial Morphology	Bacilli	Bacilli Rods	Cocci in chain	Bacilli Medium Rod	Bacilli Large Rod	Cocci in Chain	Bacillus Rods	Bacilli	Short Rods
Spore staining	Positive	Negative	Negative	Negative	Positive	Negative	Positive	Negative	Negative
Motility	Motile	Motile	Non Motile	Motile	Motile	Non Motile	Motile	Non Motile	Non Motile

Table 3: Biochemical and Enzymatic Features of Bacterial Isolates

Biochemical Tests	B1	B2	B3	B4	B5	B6	B7	B8	B9
Catalase	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive	Negative
Oxidase	Variable	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
Coagulase production	NA	Negative	Negative	NA	NA	Negative	NA	NA	Negative
O-F Test	NA	F	F	O	F	NA	NA	F	F
Habitat*	A/FAn	FAn	FAn	OAn	An	An	A/FAn	FAn	An
Hemolysis	Positive	Negative	Positive	Positive	Negative	Variable	Positive	Negative	Negative
H₂S production	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Variable
Urease activity	Negative	Negative	Negative	Negative	Negative	Negative	NA	Positive	Negative

Nitrate Reduction	Negative	Positive	NA	Positive	Negative	Positive	Variable	Positive	Negative
Indole Production	Negative	Positive	NA	Negative	Negative	Negative	Negative	Negative	Variable
Methy red Test	Negative	Positive	NA	Negative	Negative	NA	Negative	Negative	Negative
Voges Proskaur test	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Negative
Citrate Utilization test	Positive	Negative	NA	Positive	Positive	Negative	Positive	Positive	Negative

*A/Fan-aerobic/facultative anaerobic An-anaerobic FAn-facultative anaerobic NA-not applicable OAn-obligate anaerobic F-fermentative O-oxidative

Table 4: Sugar Fermentation Results of Bacterial Isolates

Sugar Fermentation	B1	B2	B3	B4	B5	B6	B7	B8	B9
Glucose	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive
Fructose	Positive	NA	Positive	NA*	Positive	Negative	Positive	NA	Positive
Arabinose	Positive	Positive	Negative	NA	Negative	Negative	Negative	Positive	Negative
Galactose	Positive	NA	Positive	NA	Negative	Variable	Negative	NA	Positive
Arabitol	Negative	NA	Negative	NA	Negative	Negative	Negative	Positive	NA
Dulcitol	Negative	NA	Negative	NA	Variable	Negative	NA	NA	Negative
Maltose	Positive	NA	Positive	NA	Negative	Negative	Positive	Positive	Positive
Sucrose	Positive	Positive	Positive	Negative	Negative	Negative	Variable	Positive	Positive
Xylose	Positive	NA	Negative	NA	Negative	Negative	Negative	Positive	Negative
Lactose	Negative	Positive	Positive	Negative	Negative	Negative	Positive	Positive	Positive

*NA-not applicable

ISSN (Online) : 2348 - 2001

International Refereed Journal of Reviews and Research

Volume 5 Issue 6 November - December 2017

International Manuscript ID : 23482001V5I6112017-04

(Approved and Registered with Govt. of India)

Registered with Council of Scientific and Industrial Research, Govt. of India

Registered in UGC Approved List of International Journals

References

Gagandeep, Kaur; Y.S. Brar and D. P. Kothari; (2017), "Potential of Livestock Generated Biomass: Untapped Energy Source In India" *Energies* Vol 10(7): 847.

Lovely.D; (2006), "Microbial Fuel Cells: Novel Microbial Physiologies and Engineering Approaches," *Current Opinion in Biotechnology*,17, pp.327-332.

Rahimnejad, M., Adhami, Arash., Darvari, Soheil., (2015)," Microbial Fuel Cell as New Technology for Bioelectricity Generation: A Review," *Alexandria Engineering Journal*, 54, pp.745-756.

Nene (1999), "Utilizing Traditional Knowledge in Agriculture," *Traditional Knowledge of India and Sri Lanka* PP 32-38.

Kartikey, Gupta., Aneja, K.R., Rana, D.,(2016),"Current Status of Cow as a Bioresource for Sustainable Development," *Bioresource and Bioprocessing*, 3(28), pp.1-11.

Davis, J.B., and Yarbrough, H.F., (1962), "Preliminary Experiments on Microbial Fuel Cells," *Science*, 116, pp.615-616.

Bruce, E.logan., Bert, Hamelers., Rene, Rozendal., UWE, Schroder., Jurg, Keller., Stefano, Freguia., Peter, Aelterman., Willy, Verstraete., Korneel, Rabaey.,(2006) "Microbial Fuel Cells: Methodology and Technology," *Environmental Science & Technology*, 40(17), pp.5181-5190.

Heilmann, and Logan, E. Bruce., (2006),"Production of Electricity from Proteins using Microbial Fuel Cells," *Water Environment Research*, 78(5), pp.115-131.

Frazier and Westhoff (1995) "Medium of Environment and Isolation of Bacteria From an Organic Waste Digester. Page 122-130.

Talaro, P. K; (2009), "Foundation in Microbiology", San Francisco: Pearson Benjamin Cummings.

Bergey's Manual Of Determinative Bacteriology, 7th edition by Bergey and Buchanan, R.E. Teo, K.C and Teoh, S. M; (2011), "A Preliminary Screening Of Microbes Isolated from Cow Dung In Kampar" *African Journal of Biotechnology*. Vol 10(9) page 1640-1645.

Dhadse, Sharda., Kankal, N.C., Kumari., Bharti.; (2012), " Study of Diverse Methanogenic and Non-Methanogenic Bacteria used for the Enhancement of Biogas Production," *International Journal of Life sciences Biotechnology and Pharma Reserch*, 1(2), pp. 176-191.

Yuti, Ishikava and Machito, mihara; (2013) "Changes In Surviving Microorganism in Cow Manure Adding Lime Nitrogen" *International journal of environment and rural development*. 4-2.

Adegunloye, D.V., F.C. Adetuyi., F.A. Akinyosoye, M.O. Doyeni., (2007), " Microbial Analysis of Compost using Cow dung as Booster," *Pakistan Journal of Nutrition*, 6(5), pp. 506-510.

Gopinath, L.R., P. Merlin Christy, K. Mahesh, R. Bhuvaneswari, D. Divya.; (2014), " Identification and Evaluation of Effective Bacterial Consortia for Efficient Biogas Production," *IOSR Journal of Environmental Science, Toxicology and Food Technology* (IOSR-JESTFT), 8(3), pp. 80-86.

Shiv, kumar; Harash, Dev, Kumar; Ghireesh, Babu K; (2012), "A Study on Electrical Generation from Cow Dung Using Microbial Fuel Cells" *Journal of biochem technology* 3(4):442: 447.

Sawant, A.A; Heyde, N.V; Straley, B.A; Donaldson, S.C; Love, B.C; Kanabel, S.J; Jayaroo, B.M; (2007), "Antimicrobial Resistant Enteric Bacteria from Dairy Cattle" *Applied Environmental Microbiology* 73: 156-163.

Prakash, Anand., (2016), “ Microbial Fuel Cells: A Source of Bio-energy,” Journal of Microbial and Biochemical Technology, 8(3), pp.247-255.