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DOES AND DONT DOES MICROBIAL FUEL CELL

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ABSTRACT

Four samples, dairy waste water, municipal waste water, textile effluent and cow dung slurry were collected from Sangli (M.H) as a substrate in MFC. Firstly, these samples were analyzed with different parameters such as pH, temperature, TDS, TSS, TS, BOD and COD before use and after use. Then from this four samples isolation and identification of bacteria is carried out by referring Bergeys manual of bacteriology. At last all four samples, isolated and identified pure and mixed bacterial cultures were used for waste treatment and bioelectricity generation in MFC.

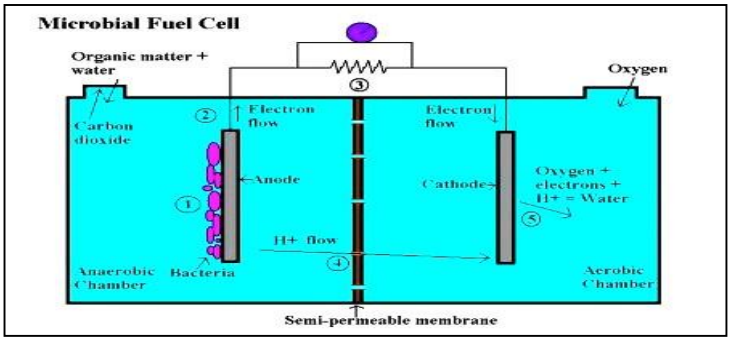
In results, 90%, 85%, 95% and 88% BOD removed from dairy waste water, municipal waste water, textile effluent and cow dung slurry respectively as a substrate after 10 days of incubation in MFC. 88%, 92%, 95% and 75% COD removed from dairy waste water, municipal waste water, textile effluent and cow dung slurry respectively as a substrate after 10 days of incubation in MFC respectively.

Maximum voltage generation reported as 753mV, 264mV, 426mV and 423mV from dairy waste water, municipal waste water, textile effluent and cow dung slurry respectively as a substrate after 10 days of incubation in MFC. Escherichia coli, Pseudomonas species, Bacillus species and Streptococcus species were isolated and identified from four samples and used for bioelectricity generation. Among all these Pseudomonas species generates 6.8mV high bioelectricity on 6th days of incubation period with $K_2Cr_2O_7$ anodic solution while mixed bacterial culture generates 8.5mV on 6th days of incubation with $K_2Cr_2O_7$ anodic solution. This study report, treated and untreated samples indicates that MFC is useful for the significant reduction of pollutant concentration and importantly bioelectricity generation.

KEYWORDS- *Waste samples, TS, BOD, COD, pure and mixed bacterial culture, MFC.*

INTRODUCTION:

Now a days the global warming and CO_2 emission increased due to which energy generation becomes serious environment issue [Sriram et.al; 2015]. In another hand, because of industrialization tons of solid and liquid waste is generated and thrown out in environment without any treatment which leads to pollution, new diseases and different environmental issues. Use of renewable energy sources biomass is a promising solution to overcome these problems [Bennetto et.al; 1984]. Renewable energy sources often provide energy in four areas such as electricity generation, air, and water heating/ cooling, transportation and off grid energy services. Among this, electricity generation is the primary energy source but mainly it creates crises in society due to low availability



of water and high requirement. To overcome this problem we found Microbial fuel cell as a alternative energy source.

In this present research, use of waste water, pure and mixed bacterial culture for the production of electrical energy in MFC is carried out. MFC is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganism. MFC consists of anode and cathode chamber separated by salt bridge. A microbe in the anode oxidizes fuel and the resulting electrons and protons are transferred to the cathode through the circuit and bridge respectively. MFC are self-sustaining process because bacteria utilizes nutrients from provided waste water or from provided nutrient medium and continuously produces electricity as long as there is a food source to nourish them.

MFC is widely applied in following area such as Brewery Wastewater Treatment, Sewage Treatment, Hydrogen Production, Generation of Energy out of Bio-waste/ Organic Matter, Remote Power Source, Omission of gas treatment, Sludge production, Microbial Fuel Cell generating electricity from wastewater, MFC for wastewater treatment: heavy metal removal, sewage sludge treatment, and its potential application in wastewater reuse in irrigation.

MFC development is still with challenges such as system up and further improvement of electric energy [Kumar *et.al*; 2012]. However use of MFC for practical waste water treatment is not straight forward yet because of the many remaining technical and economic issues [Chang: *et,al*;2010].

MATERIALS AND METHODS

Materials: Standard Potassium Dichromate (0.25N), Sulphuric Acid, Standard Ferrous Ammonium Sulphate (FAS) (0.1N), Ferroin indicator, Phosphate buffer solution, magnesium Sulphate solution, Calcium Chloride Solution, Ferric Chloride solution, Manganese sulphate solution, alkali iodide acid solution, Standard Thio Phosphate solution, Starch indicator and sulphuric acid, Grams staining reagents etc.

Methods: Characterization of samples with pH, temperature, conductivity, TDS, TSS, TS, standard Reflux method, BOD_5 days incubation method, Serial dilution method, spread plate method.

(A) Sample collection and storage

Dairy waste water, municipal waste water, textile effluent and cow dung slurry were collected from different regions of Sangli (M.S) in sterile bottle and stored in refrigerator.

(B) Characterization of samples before use and after use

(a) Determination of pH value

The pH value of samples was determined by using calibrated pH meter by dipping the electrode in the sample solution.

(b) Determination of Temperature value

The temperature value of samples was determined using thermometer by dipping it in the sample solution.

(c) Conductivity determination

Conductivity of samples was determined using conductivity meter by dipping the electrodes in the sample solution.

(d) Determination of Total Dissolved solids(TDS)

100 ml of the sample solution was filtered through whatman filter paper. Filtrate was evaporated and dried in oven at 100°Cfor an 1hrs.. After cooling in desiccators the amount of TDS was determined by using formula:

$$\text{TDS (mg/L)} = \frac{(A-B) \times 1000 \times 1000}{\text{Volume of sample (ml)}}$$

Where,

A = Weight of dried residue and dish (g)

B = Weight of dish (g)

e) Total Suspended Solids (TSS)

100 ml of the sample solution was filtered through whatman filter paper. Carefully removed filter paper from filtration assembly and dried in oven at 100°C for 1 hrs. After cooling in desiccators the total solid suspend were determined by using following formula:

$$\text{TSS (mg/L)} = \frac{(A-B) \times 1000 \times 1000}{\text{Volume of sample (ml)}}$$

Where,

A = Weight of dried residue on filter paper (g).

B = Weight of filter paper (g).

(f) Determination of COD

100 ml sample solution was relaxed with excess of $K_2Cr_2O_7$ in concentrated H_2SO_4 for 2 hrs, and then $AgSO_4$ were added as a catalyst. After 2 hrs whole sample solution were titrated against ferrous ammonium sulphate using ferroin indicator.

COD was counted by using following formula.

$$\text{COD (mg/L)} = \frac{(B-S \times N \times 8 \times 1000)}{\text{ml of sample.}}$$

Where,

B= ml of F.A.S consumed for blank

S= ml of F.A.S consumed for sample

N= Normality of F.A.S

(g) Determination of BOD

The 100 ml sample is diluted with 100 ml of oxygen saturated water containing nutrients and incubated at 20°C for 5 days in dark. The dissolved oxygen concentration is determined before and after incubation by using following formula.

DO (mg/ L) = (0.2 ×1000) ml of Thio sulphate + 200

Then

BOD is calculated by taking difference using following formula.

BOD (mg/L) = (D-B) × Dilution factor

Where;

D = Dissolve oxygen of 0 days – Dissolve oxygen of 5 days.

B= Dissolve oxygen of blank of 0 days - Dissolve oxygen of blank samples after 5 days.

(C) Isolation and identification of microorganisms

Serial dilution and spread plate method were used for the isolation of microorganisms. Serially diluted samples separately spreaded on sterile nutrient agar (NA) plates and incubated at $25^{O}C$, $30^{O}C$, $35^{O}C$ and $40^{O}C$ for 48hrs.

Identification of isolated microorganisms are carried out by using selective medium with reference to Bergeys manual of determinative Bacteriology.

(D) Anode solution

(a) Use of Samples: 100ml of dairy waste water, municipal waste water, textile effluent and cow dung slurry were used separately for the bioelectricity generation.

(b) Pure cultures

(i) *Escherichia coli*: 24hrs. Old culture of ***Escherichia coli*** was inoculated in 100ml of nutrient broth with 1% glucose which contains 5ml 0.04% w/v methylene blue.

(ii) *Pseudomonas species*: 24hrs. Old culture of ***Pseudomonas species*** was inoculated in 100ml of nutrient broth with 1% glucose which contains 5ml 0.04% w/v methylene blue.

(iii) *Bacillus species:* 24hrs. Old culture of ***Bacillus species*** was inoculated in 100ml of nutrient broth with 1% glucose which contains 5ml 0.04% w/v methylene blue.

(iv) Streptococcus species*:** 24hrs. Old culture of ***Streptococcus species was inoculated in 100ml of nutrient broth with 1% glucose and 2% salts which contains 5ml 0.04% w/v methylene blue.

(iv)Mixed culture: 24hrs. Old culture of ***Escherichia coli, Pseudomonas species, Bacillus species and Streptococcus species*** was inoculated in 100ml of nutrient broth with 1% glucose and 2% salts which contains 5ml 0.04% w/v methylene blue.

E) Cathode solution:

100ml of $K_2Cr_2O_7$ solution.

Results

The results of dairy waste water, municipal waste water, textile effluent, cow dung slurry, pure and mixed bacterial culture is shown as below.

1. Characterization of samples

Table I: Results of Dairy waste water sample.

Sr.No	Parameters	Before BEP	After BEP
1	PH value	7.4	6.6
2	Temperature(^{O}C)	34	38
3	TDS(mg/L)	549	195
4	TSS(mg/L)	251	86
5	TS(mg/L)	800	281
6	BOD (mg/L)	448	94
7	COD (mg/L)	765	124

Where,
BEP= Bioelectricity production, pH=power of hydrogen, TDS= Total Dissolved Solids, TSS= Total Suspended Solids, TS= Total Solids, BOD= Biological Oxygen Demand, COD= Chemical Oxygen Demand.

Table II: Results of Municipal waste water

Sr.No	Parameters	Before BEP	After BEP
1	PH value	7.3	6.8
2	Temperature(^{O}C)	22	29
3	TDS(mg/L)	394	116
4	TSS(mg/L)	365	104
5	TS(mg/L)	759	220
6	BOD (mg/L)	214	54
7	COD (mg/L)	382	84

Table III: Results of Textile Effluent

Sr.No	Parameters	Before BEP	After BEP
1	PH value	7.9	9.6
2	Temperature(^{O}C)	44	58
3	TDS(mg/L)	800	350
4	TSS(mg/L)	422	128
5	TS(mg/L)	1222	478
6	BOD (mg/L)	242	12
7	COD (mg/L)	464	52

Table IV; Results of Cow dung Slurry

Sr.No	Parameters	Before BEP	After BEP
1	PH value	6.4	5.6
2	Temperature(^{O}C)	12	27
3	TDS (mg/L)	84	34
4	TSS(mg/L)	68	18
5	TS(mg/L)	152	52

6	BOD (mg/L)	1100	180
7	COD (mg/L)	2200	400

The pH values of all samples are varied from 6 to 8 before use but this range get changed from 6 to 10 after use. It indicates that whatever microorganisms are present in the sample they utilized all nutrients during their log and stationary phase for the generation of electricity via metabolism at their optimum pH. Due to changed pH, microorganisms loses their metabolic activity which leads to low proton generation and simultaneously affects on electricity generation that is why after 6^{th} days of incubation, graph get decreased and microorganisms entered in decline phase.

The temperatures of all samples are varied from 10^{O}C to 50^{O}C before use but this range get changed from 20^{O}C to 70^{O}C after use. only Thermophiles are able to survive at this high temperature rather than psychrophiles and mesophiles.Upto 6^{th} days of incubation, temperature was below 40^{O}C therefore psychrophiles and mesophiles generates high protons and high electricity but after 6^{th} days of incubation water loses oxygen holding capacity and ultimately dissolved oxygen get decreased due to high temperature, into psychrophiles and mesophiles entered in decline phase which affects on the conductivity that is why graph values are decreased.

The total solids of all samples are varied from 150 mg/L to 1300mg/L before use but this range get changed from 50 mg/L to 500mg/L after use. It indicates that, whatever microorganisms are present in the sample they utilized all nutrients during their log and stationary phase for the generation of electricity via metabolism due to which total solid concentration get decreased but after 6^{th} days of incubation they don't get any nutrients so they entered in decline phase. In another hand this decreased TS indicates that MFC can be used for waste water treatment.

The BOD and COD of all samples are varied from 200mg/L to 2200mg/L before use but this range get changed from 10 mg/L to 400mg/L after use. High BOD and COD affects on the sustainable development of environment. before use, samples was with high BOD and COD meaning, high amount of pollutants present in the waste water but after use both BOD and COD of all samples decreased because of MFC. So MFC can be used in treatment of waste water specially removal of BOD and COD.

2. Isolation and Identification of microorganisms:

Well grown and isolated microbial colonies on Nutrient Agar medium were picked and sub cultured on selective media by making dilutions. Results are as follows.

(A) On Nutrient Agar Medium

(a) Morphology of Isolated culture.

Size (mm)	shape	color	margin	elevation
0.5	circular	white	entire	convex

(b) Microscopic features of isolated culture

Grams nature	Motility
Grams negative	motile

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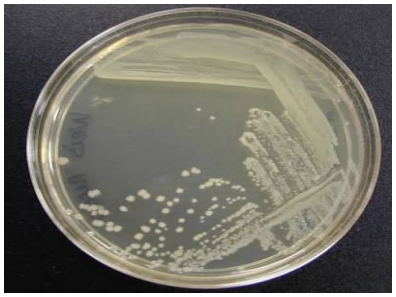
Plate I: Isolation of *Escherichia coli*

(c) Biochemical characteristics of isolated culture.

Sr.No.	1	2	3	3	5	6	7	8
Test	Indole	M-R	V-P	Citrate	sucrose	lactose	glucose	Hydrogen sulfide
Results	+	+	-	-	+	+	+	-

The morphological, microscopical and biochemical characterization of isolated culture were studied as per bergeys manual of systematic bacteriology and it was confirmed that the isolated culture was *Escherichia coli*.

(B) On Hicrome Bacillus Agar



(a) Morphology of isolated cultures

Size (mm)	shape	color	margin	elevation
0.4	circular	Yellowish	entire	convex

(b) Microscopic features

Grams nature	Motility
Grams positive	motile

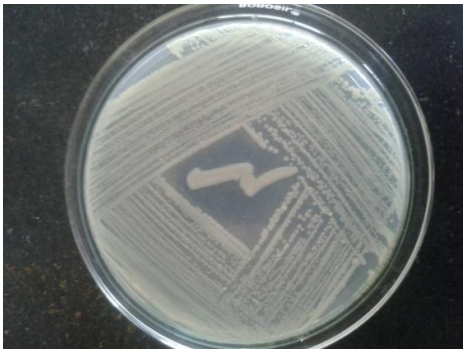
Plate II: Isolation of *Bacillus species*

(c) Biochemical characteristics of isolated culture.

Sr.No.	1	2	3	3	5	6	7
Test	Indole	M-R	V-P	Citrate	fructose	lactose	glucose
Results	-	-	+	+	+	-	+

The morphological, microscopical and biochemical characterization of isolated culture were studied as per bergeys manual of systematic bacteriology and it was confirmed that the isolated culture belongs to genus *bacillus*.

(C) On Cetrimide agar medium



(a) Morphology of Isolated culture.

Size (mm)	shape	color	margin	elevation
0.5	circular	Whitish	entire	convex

(b) Microscopic features of isolated culture.

Grams nature	Motility
Grams negative	motile

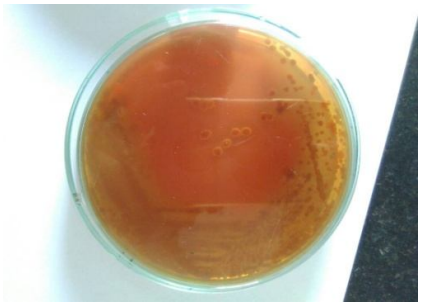
Plate III: isolation of *Pseudomonas species*

(c) **Biochemical characteristics of isolated culture.**

Sr.No.	1	2	3	3	5	6	7	8
Test	Indole	M-R	V-P	Citrate	maltose	lactose	glucose	pigmentation
Results	-	-	-	+	-	-	-	+

The morphological, microscopical and biochemical characterization of isolated culture were studied as per bergeys manual of systematic bacteriology and it was confirmed that the isolated culture belongs to genus *pseudomonas*.

(C) On Blood Sheep Agar medium



(a) **Morphology of Isolated culture**

Size (mm)	shape	color	margin	elevation
0.6	irregular	Greenish	entire	raised

(b) **Microscopic features of isolated cultures**

Grams nature	Motility
Grams positive	Non motile

Plate IV: isolation of *Streptococcus species*

(c) **Biochemical characteristics of isolated culture.**

Sr.No.	1	2	3	3	5	6	7	8
Test	Indole	M-R	V-P	Citrate	maltose	lactose	glucose	Nitrate reduction
Results	-	+	+	+	+	+	+	+

The morphological, microscopical and biochemical characterization of isolated culture were studied as per bergeys manual of systematic bacteriology and it was confirmed that the isolated culture belongs to genus *Streptococcus*.

(c) Bioelectricity generation

For the bioelectricity generation $K_2Cr_2O_7$ were used in cathodic chamber and following samples were used in anodic chamber.

Table I: Results of Dairy waste water sample: Dairy waste water contains raw milk, raw chesse, raw paneer and other waste byproducts containing carbohydrates which is not easily degradable by microorganisms in their lag or log phase means up to 4th days of incubation that is why On 4th days of incubation microorganisms entered in stationary phase and they got all optimum conditions for their growth therefore they generated **753mV** bioelectricity rather than other days**.**

Days	1	2	3	4	5	6	7	8	9	10
Voltage generated (mV)	154	248	428	753	172	147	120	102	84	42

Table II: Results of Municipal waste water: Municipal waste water contains house used water used food materials, kitchen waste water, and other waste byproducts containing organic acids which is not easily degradable by microorganisms in their lag or log phase means up to 4th days of incubation that is why On 5th days of incubation period microorganisms entered in stationary phase and they got all optimum conditions for their growth and metabolism therefore they generated **264mV** bioelectricity rather than other days**.**

Days	1	2	3	4	5	6	7	8	9	10
Voltage generated (mV)	135	142	188	226	264	202	112	74	40	21

Table III: Results of Textile Effluent: Textile Effluent contains different dyes, organic acids, used water and other waste byproducts containing toxic materials which is not easily degradable by microorganisms in their lag or log phase means up to 3rd days of incubation period that is why On 4th days of incubation period microorganisms entered in stationary phase and they got all optimum conditions for their growth and metabolism therefore they generated **426mV** bioelectricity rather than other days**.**

Days	1	2	3	4	5	6	7	8	9	10
Voltage generated (mV)	124	158	246	426	384	224	122	102	84	62

Table IV; Results of Cow dung Slurry: Cow dung Slurry contains different carbohydrates, proteins, fats, and other byproducts which is not easily degradable by microorganisms in their lag or log phase means up to 3rd days of incubation period that is why On 4th days of incubation period microorganisms entered in stationary phase and they got all optimum conditions for their growth and metabolism therefore they generated **423mV** bioelectricity rather than other days**.**

Days	1	2	3	4	5	6	7	8	9	10
Voltage generated (mV)	125	188	286	423	316	276	157	102	102	96

Bioelectricity generation from Pure Bacterial cultures:

For the generation of bioelectricity *Bacillus species, Pseudomonas species, Escherichia coli and streptococcus species* were used. Following figure indicates that**,** *Escherichia coli* (6.2mV), *Pseudomonas species* (6.8mV)*, Bacillus species* (6.4mV) *and Streptococcus species* (5.2mV) generates electricity on 6th days of incubation but as compared to others *Pseudomonas species* generates high bioelectricity.

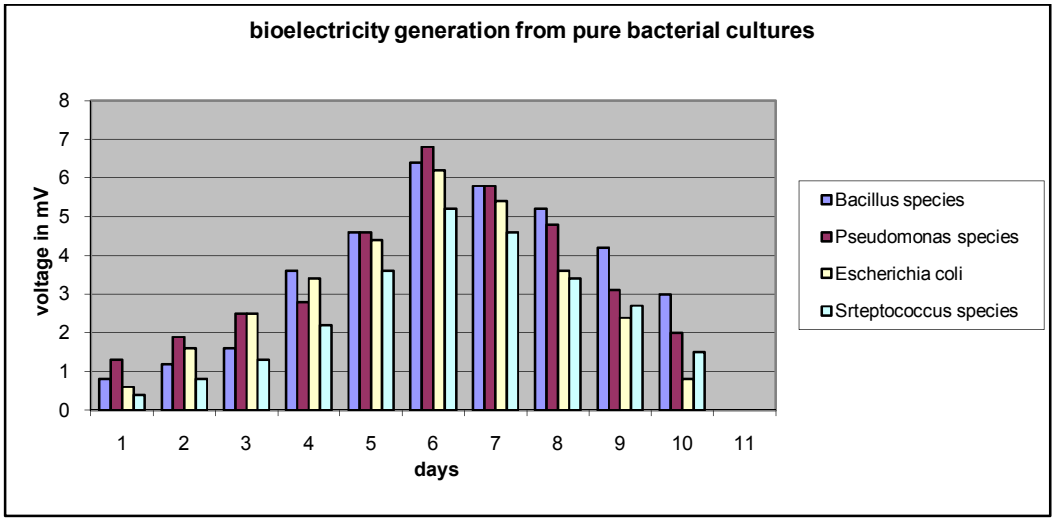


Figure I: Indicates generation of bioelectricity from pure bacterial culture

From the following figure, Up to 6th days of incubation period bioelectricity generation was slow due to microbial lag and log phase and also after 6th days of incubation period bioelectricity generation was slow due to less nutrients and death phase of microbes but on 6th days of incubation period 8.5 mV bioelectricity was generated because all enzymatic system was active and all optimum conditions was there for their growth and metabolism.

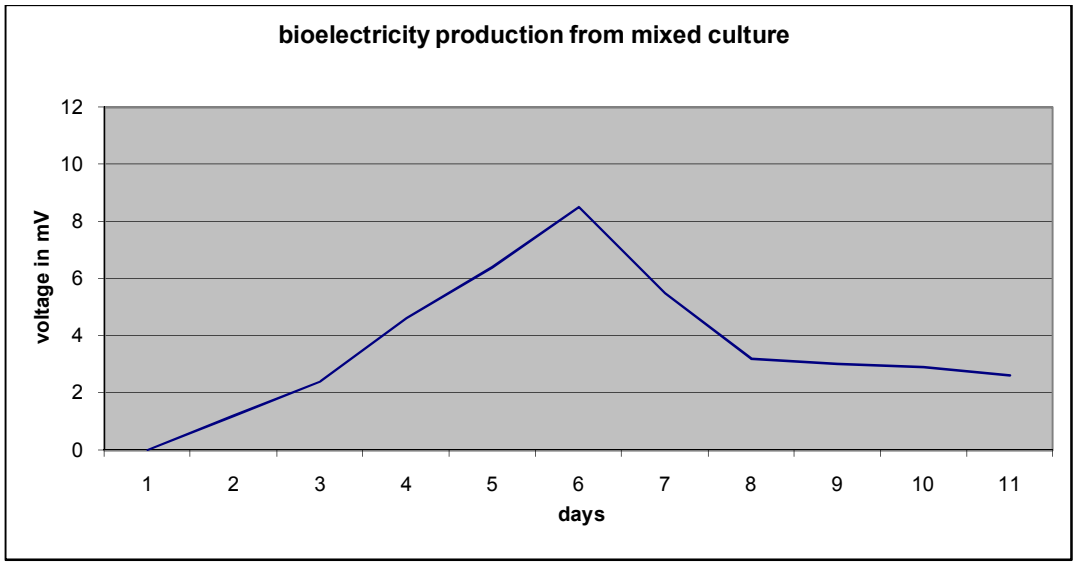


Figure II: Indicates generation of bioelectricity from mixed bacterial culture.

DISCUSSION

We designed MFC by taking PVC pipe, two plastic bottles, anode and cathode etc. and these results are coincidence with G.Buddolla *et. al;* (2015) V. Sridevi *et, al;* (2015) Bennetto *et.al;* 1984. In the Present research work we used dairy waste water, municipal waste water, textile effluent, cow dung slurry, pure and

mixed bacterial culture as like G.Buddolla *et. al;* (2015), Anand prakash, (2016), V. Sridevi *et, al;* (2015), Cheng *et al.,* (2006).

Anand prakash, (2016) reported that 599mV, 900mV, 656mVand 678mV from Agro waste, dairy waste, distillery waste and municipal waste respectively but in our study we reported 753mV, 286mV, 264mVand 426mV from dairy waste water, municipal waste water, textile effluent, cow dung slurry respectively. We got highest bioelectricity (753mV) from dairy waste water and these results are accordance with Anand prakash, (2016) because in their study they also got maximum voltage (900mV) from dairy waste water. V. Sridevi *et, al;* (2015) studied waste water for MFC in which they studied pH, temperature, conductivity, TDS, TSS, TS, COD, BOD of samples before use and after use. We also studied characterization of samples in same manner as V. Sridevi *et, al;* (2015) studied but our results are in contrast to them because their results are saying that up to 12 days all parameters may get decrease fastly due to presence of only biodegradable matter in sample but our study is saying that it is not only depends upon biodegradable matter but also depends upon presence of microorganisms in waste water sample. By using pure culture of *Shwenella Putrefaciens* Bond and Lovely, (2003) produced 33.4mW/m power density; In contrast, we obtained a maximum (6.8mV) power output from pure culture of *Pseudomonas species.* Cheng *et al.,* (2006) produced 480 mW/m using mixed cultures of microorganisms and we got 8.5mV from mixed bacterial culture..

CONCLUSION

Successfully Designed MFC model generated electricity and it is easy to maintain. This Model is also used to develop high scale production of bioelectricity. In the present research waste samples are used. By using these wastes we can generate bioelectricity which is ecofriendly. It is noted that, a group of microorganisms is able to produce more power than a single pure culture. It also proves the fact that performance of microbial fuel cells with respect to electricity generation dependent on availability of various types of microbes found in biological waste Total solids, COD, BOD removal after use indicates that microbes are involved in the biodegradation and waste water treatment therefore MFC technology is the new technique which provides a new methods for electricity generation and waste water treatment.

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