

## Treatment of Brewery Wastewater and Production of Electricity through Microbial Fuel Cell Technology

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### Abstract

Renewable energy is an increasing need in our society. Microbial fuel cells (MFCs) represent a new method for treating wastewater and simultaneously producing electricity. In the present study, we demonstrated the feasibility of bioelectricity generation from brewery wastewater treatment using a mediator less MFC at different pH. We also demonstrated that addition of readily utilizable substrates like glucose and sucrose to the wastewater can enhance the electricity production and COD removal. pH 7 was most favorable for Bioelectricity production. Up to 10.89 mA current generation and 93.8% COD removal efficiency was obtained by this method.

**Keywords:** Brewery wastewater, COD, bioelectricity, Microbial Fuel Cell.

### Introduction

World energy demand is expected to raise from 421 quadrillion British Thermal Units (BTUs) in 2003 to 563 quadrillion BTUs in 2015 to 722 quadrillion BTUs in 2030.<sup>1</sup> Energy production from renewable feed stocks holds great potential to meet these needs in a sustainable and environmentally sound manner, and to reduce dependence on fossil fuels.

At the same time, impairment of water resources requires increased investment in water and wastewater treatment infrastructure. Energy generation from “negative value” waste streams can simultaneously help meet the worlds energy needs, reduce pollution, and reduce costs associated with water and wastewater treatment.

Microbial Fuel Cells (MFCs) have drawn world wide interest as a method of

directly generating electricity from organic matter in wastewater, while simultaneously treating the wastewater.<sup>2</sup>

A microbial fuel cell (MFC) is a device that uses bacteria to catalyze the conversion of organic matter into electricity<sup>3-11</sup>. Bacteria generating electrons and protons at the anode oxidize substrate. Electrons are transferred through an external circuit while the protons diffuse through the solution to the cathode, where electrons combine with protons and oxygen to form water<sup>12-13</sup>.

Microorganisms can transfer electrons to the anode electrode in three ways: Exogenous mediators (ones external to the cell) such as Thionine, or Neutral red; using mediators produced by the bacteria; or by direct transfer of electrons from the respiratory enzymes (i.e., Cytochromes) to the electrode<sup>7, 14</sup>. These mediators trap electrons from the respiratory chain and become reduced to transfer the electron to the electrode via outer cell membrane<sup>15</sup>. *Clostridium butyricum*<sup>16</sup> *Saccharomyces cerevisiae*<sup>17</sup> and *Proteus vulgaris*<sup>15</sup> are reported to transfer electrons in mediated MFC while *Shewanella putrefaciens*, *Geobacter sulfurreducens*, *Geobacter metallireducens* and *Rhodospirillum rubrum* have been shown to generate electricity in a mediator less MFC<sup>7</sup>. Bacteria present in mediator less MFCs have electrochemically active redox enzymes on their outer membranes that transfer the electrons to external materials and therefore, do not require exogenous chemicals to accomplish electron transfer to the electrode<sup>11</sup>.

MFCs have operational and functional advantages over the technologies currently used for generating energy from organic matter. First, the direct conversion of substrate energy to electricity enables high conversion efficiency. Second, MFCs operate efficiently at ambient temperature. Third, an MFC does not require gas treatment because the off-gases of MFCs are enriched in carbon dioxide and normally have no useful energy content. Fourth, MFCs do not need energy input for aeration provided the cathode is passively aerated<sup>9</sup>. Fifth, MFCs have potential for widespread application in locations lacking electrical infrastructures and can also operate with diverse fuels to satisfy our energy requirements. Some recent developments allow high conversion rates and high conversion efficiencies of simple carbohydrates like glucose, and complex carbohydrate like starch<sup>16</sup> and cellulose<sup>17</sup>. Although MFCs generate a lower amount of energy than hydrogen fuel cells, a combination of both electricity production and wastewater treatment would reduce the cost of treating primary effluent wastewater.

When a complex wastewater was used as fuel, bio-film formed onto the anode in addition to microbial clumps loosely associated with the electrode. The microbial clumps are believed to ferment the complex fuel to simple fermentation products, which are oxidized by the electrochemically active microorganisms in the bio-film.<sup>4,5</sup>

Large amount of beer brewery wastewater is produced from cooling (eg. saccharification cooling, fermentation) and washing units in brewery industry and often causes several environmental problems. Although brewery wastewater have high Chemical Oxygen Demands (CODs) but are nontoxic because much of the organic matter in the water consists of sugar, starch, and protein.<sup>6</sup>

In the present study we used brewery wastewater for bioelectricity production.

Beer brewery wastewater have been examined earlier<sup>2</sup> as a substrate for power generation in an MFC, and found suitable for electricity generation due to the food derived nature of the organic matter and the lack of high concentration of inhibitory substances (for example, ammonia in animal wastewater). We therefore investigated the possibility of enhanced electricity production by adding glucose and sucrose in wastewater at pH 6, 7 and 8.

## Materials and Methods

**Beer Brewery Wastewater Samples:** Wastewater was collected from the Central Distilleries and Breweries Ltd. Meerut, India, and divided into nine 1 liter volumes. Each sample was left undisturbed for 24 Hrs. at 4°C under anaerobic conditions so as to settle the solid particulate contents. The 900 ml supernatant of each was taken, per analysis. Each sample was diluted 4 times, and was differentially treated, made in duplicate and designated as given below:

- Sample A: Plain diluted waste water without any treatment.
- Sample B: 10% sucrose solution of plain diluted waste water (Sample A).
- Sample C: 10% glucose solution of plain diluted waste water (Sample A).

**Chemicals:** All chemicals used in this study were of analytical or Biochemical grade.

**MFC Construction:** The MFCs were constructed from glass (16x16x10 cm) with a total volume of 1000 ml, and working volume of 700 ml. Both anode and cathode were separated by a glass, containing hole (6x6 cm) which was covered with a proton exchange membrane (Nafion™ 117, DuPont Co.). Three electrode arrangements consisting of plain carbon paper (7x7cm) as anode and graphite (7x7 cm) were used in this study. The electrodes were attached using copper wire with all exposed metal surfaces sealed with a nonconductive epoxy. The anode chamber was filled (600 mL) with various Samples respectively for separate study. The anode was continuously flushed with N<sub>2</sub>/CO<sub>2</sub> (80:20) to maintain anaerobic conditions. Cathode chamber (aerobic chamber where oxygen was used as the electron acceptor for the electrode) was filled with 100mM Phosphate Buffer and pH adjusted to 7 by 0.5 N NaOH. The cathode chamber was provided with air that was passed through a 0.45µm pore size filter.

**MFC operation:** Initially MFCs were inoculated with Artificial wastewater containing glucose as carbon source. The composition of wastewater was (per liter): 1.0 g glucose, 450.0 mg NaHCO<sub>3</sub>, 100 mg NH<sub>4</sub>Cl, 10.5 mg K<sub>2</sub>HPO<sub>4</sub>, 6.0 mg KH<sub>2</sub>PO<sub>4</sub>, 64.3 mg CaCl<sub>2</sub>.2H<sub>2</sub>O, 18.9 mg MgSO<sub>4</sub>.7H<sub>2</sub>O, 10.0 mg FeSO<sub>4</sub>.7H<sub>2</sub>O, 6 mg MnSO<sub>4</sub>, 0.5 mg ZnSO<sub>4</sub>.7H<sub>2</sub>O, 20 mg CoCl<sub>2</sub>.6H<sub>2</sub>O, 0.65 mg CuSO<sub>4</sub>.5H<sub>2</sub>O. After two cycles, feed solution containing 50% artificial wastewater and 50% different brewery wastewater Samples, separately inoculated into MFCs. After four cycles, feed solution was switched to brewery wastewater samples.

**Monitoring Electricity:** Voltage and current on Microbial Fuel Cells were measured by a Digital Multimeter (Kusam electrical industries, Model – 108). Continuous measurements at 24 Hr. interval across 10Ω resistance, were taken throughout the experiments. Current (I) was calculated as  $I(\text{mA}) = V(\text{mV})/R(\Omega)$ , where V is the

voltage and  $R$  is the external resistance. COD measurements were conducted using standard methods<sup>18</sup>. All experiments were carried out at 35°C.

**Statistical analyses:** All experiments were conducted using 3 separate microbial fuel cells. When a single MFC was used the experiments were repeated at least 3 times. And results were presented as average values or a typical result. All experimental error was analyzed with an aid of statistical methodology provided by Sigma Plot 7.1 (SPSS Inc., Chicago, IL, USA). We found that the all data presented were statistically significant.

## Result and Discussion

After setting the experiment, all two chambered Mediator Less MFCs were operated with different designated waste water Samples at various pH, as feed to support the formation of biomass and subsequent generation of electricity. Preliminary experiments conducted using MFCs showed that electricity could be generated using Brewery waste water, and that the bacteria needed for electricity generation were already present in the wastewater. Stable current output was achieved after two cycles.

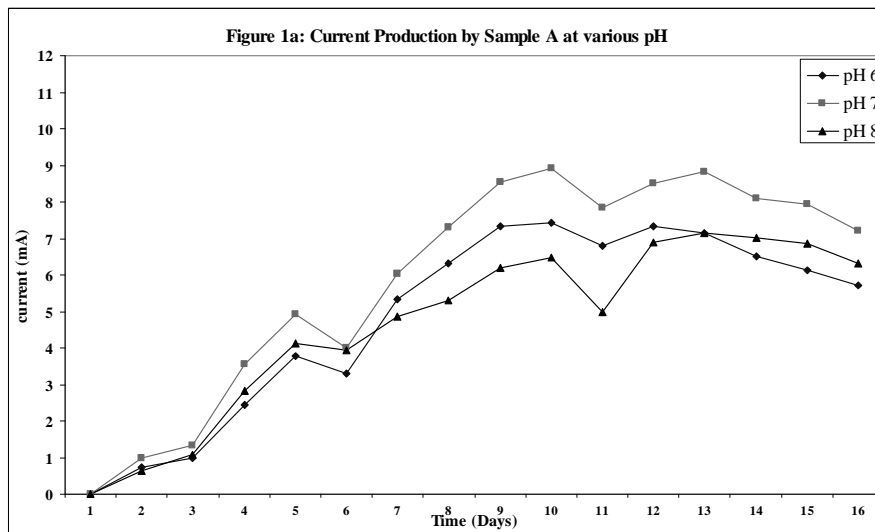
When MFCs were inoculated with different designated Brewery waste water samples, there was a 24 h Lag phase followed by an increase in cell current during next 96 h (4 days). The initial increase of current here can be attributed to the presence of components that are easily utilized by anodic bacteria. When these easily degradable substrates were exhausted, the current outputs began to decrease. Meanwhile, degradation of complex components was taken place by which a lower current was still obtained. Fresh feed was supplemented 5<sup>th</sup> day, after a drop in current was observed. A steady increase in current generation was observed with every additional feed and might be attributed to the adaptation, phenomenon and development of the biofilm on the surface of the anode. Electrode fouling was not observed and the electrodes could be used in further experiments without remarkable activity loss.

Effect of fuel and pH of anodic chamber on electricity generation: MFCs were operated with different designated wastewater samples at various pH to check their electricity generation capacity (Table 1). Figure 1a, 2a and 3a shows current generation by Sample A (Plain diluted wastewater), Sample B (10% sucrose solution of Sample A) and Sample C (10% sucrose solution of Sample A) at pH 6, 7 and 8.

At all pH, Sample A started fermentation and current generation after about 24 hrs. It reached the current output of 3.78 mA, 4.92 mA and 4.14 mA after 4 days of operation, when operated at pH 6, 7 and 8 respectively. The major feature was a drop of the current after approximately 5 days at all pH. The current generation recovered quickly when 50% part of Sample A was replaced with a syringe through the anode and reached a maximum value of 7.43 mA, 8.92 mA and 6.48 mA respectively after 9 days. After 10 days again a current drop observed which recovered after addition of fresh Sample A to the anode compartment and reached to maximum output of 7.13 mA, 8.83 mA and 7.01 mA after 12 days. Sample A showed its best performance at pH 7.

**Table 1:** Current Generation and COD removal by all Samples at different pH

	Days	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
S A M P L E A	pH 6	Current	0.00	0.74	0.97	2.45	3.78	3.29	5.33	6.31	7.33	7.43	6.78	7.32	7.13	6.51	6.13	5.71
		COD mg/L	1710	1687	1565	1456	1388	1267	1191	1135	1086	959	901	831	758	692	555	487
		% Efficiency of COD removal	0.00%	1.34%	8.47%	14.85%	18.83%	25.90%	30.35%	33.62%	36.49%	43.91%	47.30%	51.40%	55.67%	59.53%	67.54%	71.52%
	pH 7	Current	00	.98	1.33	3.56	4.92	4.01	6.03	7.31	8.53	8.92	7.83	8.52	8.83	8.11	7.93	7.21
		COD mg/L	1710	1701	1605	1229	1188	1032	971	943	709	683	545	506	428	401	293	242
		% Efficiency of COD removal	0.00%	0.52%	6.14%	28.12%	30.5%	39.6%	43.21%	44.85%	58.53%	60.0%	68.1%	70.4%	74.9%	76.5%	82.8%	85.8%
	pH 8	Current	0.00	0.65	1.09	2.81	4.14	3.93	4.87	5.31	6.18	6.48	4.99	6.89	7.13	7.01	6.87	6.31
		COD mg/L	1710	1693	1631	1571	1508	1398	1311	1210	1178	1132	1052	931	852	772	711	653
		% Efficiency of COD removal	0.00%	0.99%	4.61%	8.12%	11.81%	18.24%	23.33%	29.23%	31.11%	33.80%	38.47%	45.55%	50.17%	54.85%	58.42%	61.81%
S A M P L E B	pH 6	Current	0.00	1.21	1.63	4.31	6.13	4.83	7.89	8.87	9.32	9.01	7.89	8.86	8.71	8.83	8.43	7.87
		COD mg/L	1710	1663	1532	1457	1348	1234	1031	934	873	798	689	603	519	453	357	254
		% Efficiency of COD removal	0.00%	2.74%	10.40%	14.79%	21.16%	27.83%	39.70%	45.38%	48.94%	53.33%	59.70%	64.73%	69.64%	73.50%	79.12%	85.14%
	pH 7	Current	0.00	.89	1.32	3.61	5.83	5.49	7.36	8.10	8.01	8.96	8.67	9.31	9.59	9.53	9.40	8.91
		COD mg/L	1710	1683	1592	1302	1287	1046	1021	893	675	659	447	415	385	301	213	138
		% Efficiency of COD removal	0.00%	1.57%	6.90%	23.85%	24.73%	38.8%	40.29%	47.7%	60.52%	61.46%	73.85%	75.73%	77.48%	82.39%	87.54%	91.92%
	pH 8	Current	0.00	0.88	1.21	2.66	5.31	4.32	5.99	6.77	7.53	7.92	7.67	7.22	7.00	6.61	6.23	6.02
		COD mg/L	1710	1671	1601	1522	1489	1320	1198	1101	1013	983	832	742	653	532	483	408
		% Efficiency of COD removal	0.00%	2.28%	6.37%	10.99%	12.92%	22.80%	29.94%	35.61%	40.76%	42.51%	51.34%	56.66%	61.81%	68.88%	71.75%	76.14%
S A M P L E C	pH 6	Current	0.00	0.98	1.45	3.89	6.98	4.32	7.53	8.63	9.23	9.83	8.81	9.42	9.13	8.51	7.93	7.01
		COD mg/L	1710	1643	1512	1423	1345	1189	974	854	817	741	632	541	427	353	287	208
		% Efficiency of COD removal	0.00%	3.91%	11.57%	16.78%	21.34%	30.46%	43.04%	50.05%	52.22%	56.66%	63.04%	68.36%	75.02%	79.35%	83.21%	87.83%
	pH 7	Current	00	1.53	1.81	4.96	6.75	6.52	8.27	9.42	9.33	10.89	10.36	9.93	8.79	8.01	7.12	6.68
		COD mg/L	1710	1642	1561	1187	1152	898	841	802	643	619	476	409	334	235	228	105
		% Efficiency of COD removal	0.00%	3.9%	8.71%	30.58%	32.63%	47.48%	50.81%	53.09%	62.39%	63.80%	72.16%	76.0%	80.4%	86.2%	86.6%	93.8%
	pH 8	Current	0.00	0.91	1.13	3.44	5.57	3.92	6.53	7.41	8.57	8.83	7.89	8.52	8.23	7.97	7.43	6.11
		COD mg/L	1710	1653	1576	1498	1407	1288	1175	1054	963	897	755	687	590	513	465	389
		% Efficiency of COD removal	0.00%	3.33%	7.83%	12.39%	17.71%	24.67%	31.28%	38.36%	43.68%	47.54%	55.84%	59.82%	65.49%	70.00%	72.80%	77.25%



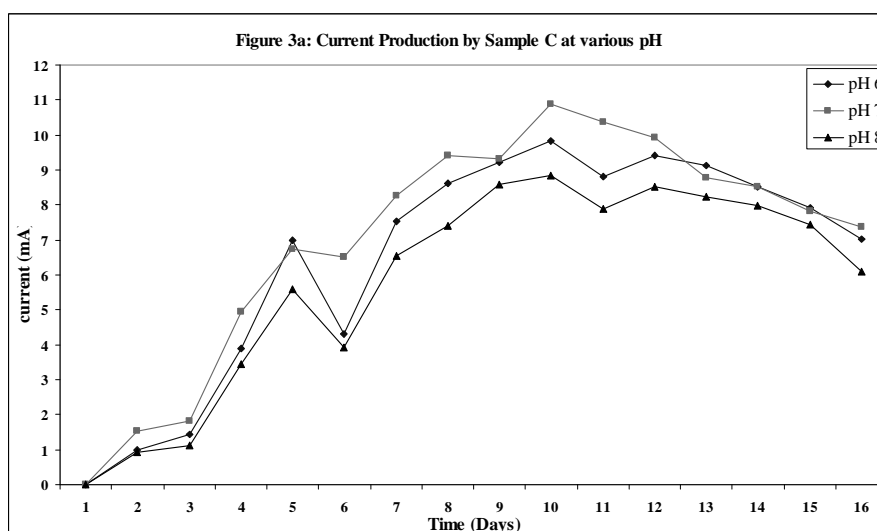
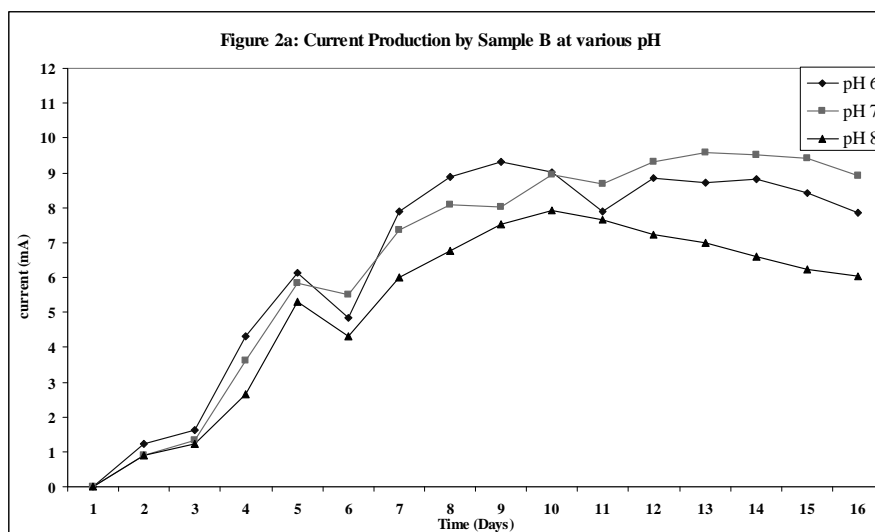


Figure 3a shows, current generation from Sample C at pH 6, 7 and 8. At all pH, Sample C started fermentation and current generation after about 24 hrs. At pH 6 it reached the maximum current output of 6.98 mA after 4<sup>th</sup> day of operation. After observing a current drop after 5<sup>th</sup> and 10<sup>th</sup> day, 50% part of Sample C was replaced with a syringe through the anode. The current generation recovered quickly after substrate feeding and reached a maximum value of 9.83 mA and 9.13 mA after 9<sup>th</sup> and 12<sup>th</sup> days. At pH 7, Sample C, also started current generation after about 24 hrs and reached maximum current output of 6.75 mA, 10.89 mA and 8.79 mA after 4<sup>th</sup>, 9<sup>th</sup> and 12<sup>th</sup> day of operation respectively. MFC operating at pH 8, also reached maximum current output of 5.57 mA, 8.83 mA and 8.23 mA after 4<sup>th</sup>, 9<sup>th</sup> and 12<sup>th</sup> day of operation respectively. Here Current drop also observed after 5<sup>th</sup> and 10<sup>th</sup> day which recovered after addition of 50% fresh feed. As shown in the figure 3a, that after

addition of 10 % glucose to the wastewater sample, increased current obtained throughout in Sample C, which may be due to readily oxidizable property of the glucose. And as it accelerated the degradation of wastewater, so even after consumption of glucose, we obtained increased current.

Figure 1a, 2a and 3a clearly indicating that current was generated in proportion to fuel concentration. At all pH Current generation by Sample B and Sample C was much larger than those obtained with Sample A. Above differences in current generation arise from substrate concentration and form (soluble or particulate), intrinsic microbial kinetics and the complexity of the microbial community in the biofilm that is need to completely degrade the substrate.<sup>19</sup>

So current generation of a MFC can be increased by adding small amount of readily utilizable substrate to the wastewater, which can accelerate the utilization of the wastewater. The MFCs with Sample C (Glucose) produced current higher than both MFCs, but the MFC containing Sample B produced an stable and maintained current over a long period of time.

Each microbial group involved in anaerobic degradation had a specific pH optimum in which it grew and performed best. As shown in figure 1a, 2a and 3a, the highest current was observed at pH 7, while values were decreased at pH 6.0 and pH 8.0. These results clearly indicate that generally microbial activity is lower at sub-optimal pH than an optimal pH. Low value at pH 8.0 might be due to poor proton transfer at the reduced proton concentration gradient across the membrane.<sup>20</sup>

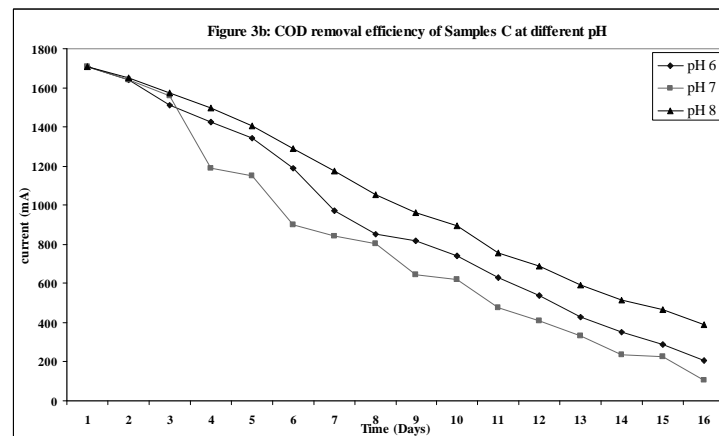
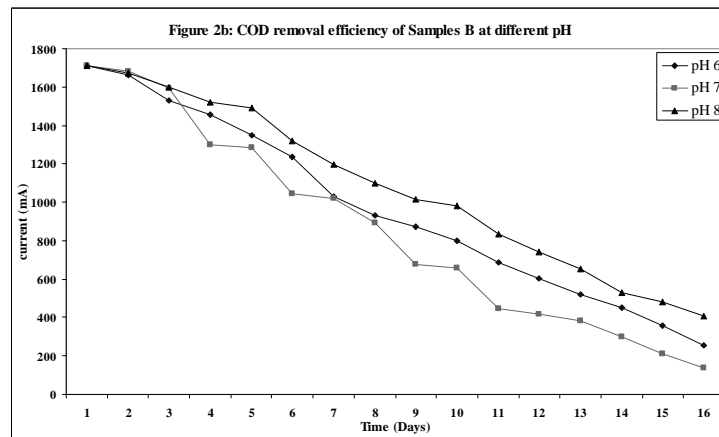
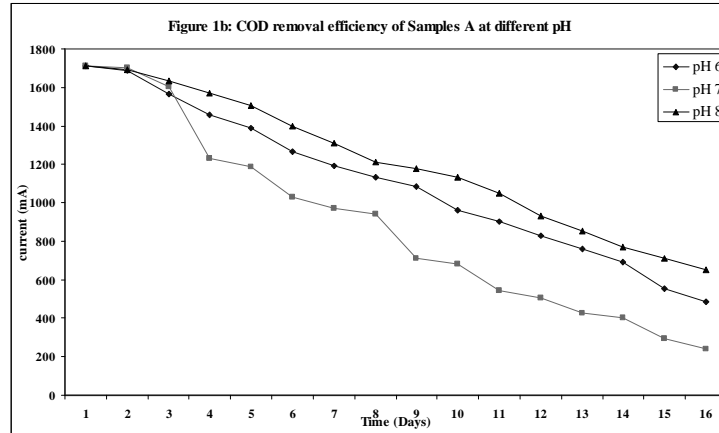
#### **Effect of fuel and pH of anode compartment on COD removal:**

During above operation, the MFCs were continuously monitored for COD removal efficiency. The MFCs were operated at influent COD concentration of 1710 mg/L. Figure 1b, 2b, 3b and Table 1 shows COD removal efficiency of Sample A (Plain diluted wastewater), Sample B (10% sucrose solution of Sample A) and Sample C (10% sucrose solution of Sample A) at pH 6, 7 and 8 respectively.

It is evident from experimental data that substrate feeding rate documented its marked influence on current yield and substrate degradation, and it was observed that current generation and COD removal show relative compatibility. Continuous COD removal was observed in all MFCs. COD removal efficiency increased each time with substrate feeding. This may be due to availability of readily utilizable carbon sources to microorganisms present in wastewater.

After 15 days of operation, the COD removal efficiency of MFCs containing Sample A, Sample B and Sample C was 85.8%, 91.92% and 93.8% respectively at pH 7. As shown in Table 1, relatively higher COD removal efficiency and substrate degradation rate was observed with Sample B and Sample C than Sample A.

Maximum COD removal efficiency was exhibited by Sample C, which was 10% glucose solution of plain diluted wastewater, then by Sample B which was 10% sucrose solution of Plain wastewater. Glucose and sucrose present in these samples were quickly utilized by microbes and they accelerated the degradation of other carbon sources present in the wastewater. As glucose is readily metabolizable than sucrose, so the Sample C was exhibited best performance in COD removal.



Time taken for substrate exhaustion was relatively more in Sample A which caused lower COD removal efficiency. Lower COD removal efficiency observed in this case could be attributed to unavailability of readily oxidizable carbon sources in Sample A for the degradation and production of electricity. pH strongly affect microbial activity, and it is evident from data that microorganisms present in brewery wastewater were more active at pH 7 than pH 6 and pH 8 for electricity generation by

COD removal.

Decrease in COD concentration, increase in COD removal efficiency and simultaneous current production indicated effective functioning of the selectively enriched mixed microflora in metabolizing the carbon source present in wastewater as electron donors. It is evident from experimental data that the MFC acted as suspended growth bioreactor used for wastewater treatment with respect to COD removal efficiency along with power generation.

## **Conclusion**

Although Brewery wastewater is not ideal substrate for electricity generation in MFCs as its insolubility and chemical stability limits the rate of microbial substrate decomposition and thus the current output that can be achieved, yet it provides good results with readily oxidizable substrates like glucose and starch. Major issues to be solved for practical application are to overcome the activity loss and incomplete utilization of wastewater. If power generation in these systems can be increased, MFC technology may provide a new method to offset wastewater treatment plant operating cost, making wastewater treatment more affordable for developing and developed nations. Thus, the combination of wastewater treatment along with electricity production may help in saving money as a cost of wastewater treatment at present.

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