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Bioelectricity Production by *Saccharomyces cerevisae* from Sugar Industry Waste using Microbial Fuel Cell Technology

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Microbial fuel cells (MFCs), that remove carbon as well as nitrogen compounds out of wastewater are of special interest for practice. In a microbial fuel cell (MFC), power can be generated from the oxidation of organic matter by bacteria at the anode, with reduction of oxygen at the cathode. Proton exchange membranes used in MFCs are permeable to oxygen, resulting in the diffusion of oxygen into the anode chamber. In this contribution we demonstrate electricity production by Sugar Industry Waste Water using *Saccharomyces cerevisae.* By combining Methylene Blue, a greater current generated from waste water. Up to 10.45 mA Current was achieved in 10 days of operation.

Key words: Bioelectriciy, Methylene Blue, Microbial Fuel Cells, *Saccharomyces cerevisae*, Sugar Industry waste.

Wastewater treatment has begun to be focused on waste reuse and energy recovery due to environmental concerns and finite fossil fuels[1]. India is one of the largest producers of sugar in the World, sugar mills generate about 1,000 liters of wastewater for per tone of cane crushed, and the effluent is mainly floor washing wastewater, sugarcane juice, syrup and molasses with a BOD of 1,000-1,500 mg/l.

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The sugar mill waste water contains large quantities of bio-degradable organic matter and therefore biological treatment processes are most commonly used for its treatment. In general, anaerobic biological processes (oxidation ponds and biomethanation) have several advantages over aerobic processes (aerated lagoons, activated sludge process). Anaerobic processes decompose the organic compounds in an atmosphere free of oxygen and consequently require significantly less energy as compared to aerobic processes. As compared to aerobic processes, anaerobic processes are easier to control and operate, produce a lower quantity of sludge and their annualized costs are lower. So, it is preferable to treat sugar mill waste waters by anaerobic processes rather than aerobic processes. Treated waste water with a BOD level of about 100 mg/l can be used for irrigation of sugar cane fields. Microbial Fuel Cells (MFCs) are a relatively new

technique in wastewater treatment which converts organic matter into electricity[2-5].

Microbial fuel cells (MFC) are electrochemical devices that convert the chemical energy contained in organic matter into electricity by means of the catalytic (metabolic) activity of living microorganisms[6-9]. An MFC consists of anode and cathode separated by a cation specific membrane. In the anode compartment of an MFC microorganisms oxidize fuel (substrate) generating electrons and protons. 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[10]. Oxygen is superior to other electron acceptors for its unlimited availability and its high redox potential[11].

$$4H^+ + 4e^- + O_2 \rightarrow 2H_2O$$

OR

$$4H^+ + 4e^- - + 2O_2 \rightarrow 2H_2O_2$$

Microorganisms can transfer electrons to the anode electrode in three ways: Exogenous mediators (ones external to the cell) such as Methylene Blue [6] or Neutral red [4]; using mediators produced by the bacteria; or by direct transfer of electrons from the respiratory enzymes (i.e., Cytochromes) to the electrode[12,13]. These mediators trap electrons from the respiratory chain and become reduced to transfer the electron to the electrode via outer cell membrane[14]. *Clostridium butyricum*[15] *Saccharomyces cerevisae*[16] and *Proteus vulgaris*[14] are reported to transfer electrons in mediated MFC while *Shewanella putrefaciens*[17], *and Geobacter sulfurreducens*[18], have been shown to generate electricity in a mediator less MFC[10]. 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[19].

In the present study we demonstrate a Microbial Fuel Cell powered by "*Saccharomyces cerevisae*" to treat Sugar Industry Wastewater. *Saccharomyces cerevisae* (Yeast) is heterotrophic, found in a wide range of natural habitat, grow rapidly under both aerobic and anaerobic conditions, have simple nutritional requirements and can utilize a wide variety of substrates which make them ideal for a MFC[20].

MATERIALS AND METHODS

Sugar Industry Wastewater

Wastewater sample was collected from nearby Sugar Industry and divided into four volumes. Each sample was left undisturbed for 24 Hrs. at 35ºC under anaerobic conditions so as to settle the solid particulate contents. The 700 ml supernatant of each was taken, per analysis. Each sample was differentially treated, made in duplicate and designated as below:

Medium A1: Plain 2 times diluted wastewater.
Medium A2: 0.5% Methylene Blue solution of plain 2 times diluted wastewater (Medium A1).
Medium B1: Plain 4 times diluted wastewater.
Medium B2: 0.5% Methylene Blue solution of plain 4 times diluted wastewater (Medium B1).

Chemicals and microorganism

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

Microbial seeds of *Saccharomyces cerevisae,* available in the laboratory used in present study. *S. cerevisiae* was maintained at 4ºC on agar slants. The composition of the agar slant was (g l^{-1}): 3.0 yeast extract, 3.0 malt extract, 5.0 peptone, 10.0 glucose and 20.0 agar. The cultures were maintained by sub-culturing every 20-days and the test tubes were then incubated at 35 °C for 36 h.[21] The medium was adjusted to a pH of 7.0. 15% inoculum of this culture was used to transfer into anode chamber of MFC for electricity production study.

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) as cathode 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 Mediums respectively for separate study. The anode was continuously flushed with N_2/CO_2 (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 (g l^{-1}): 1.0 g glucose, 450.0 mg $NaHCO_3$, 100 mg NH_4Cl, 10.5 mg K_2HPO_4, 6.0 mg KH_2PO_4, 64.3 mg $CaCl_2.2H_2O$, 18.9 mg $MgSO_4.7H_2O$, 10.0 mg $FeSO_4.7H_2O$, 6 mg $MnSO_4$, 0.5 mg $ZnSO_4.7H_2O$, 20 mg $CoCl_2.6H_2O$, 0.65 mg $CuSO_4.5H_2O$. After two cycles, feed solution containing 50% artificial wastewater and 50% different Sugar Industry wastewater Mediums, separately inoculated into MFCs. After four cycles, feed solution was switched to various Sugar Industry wastewater Mediums.

Monitoring Electricity

Current (*I*) measurements were recorded using a Digital Multimeter (Kusam electrical industries, Model – 108) by connecting with 10Ω external circuit. COD measurements were conducted using standard methods[22].

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. We found that the all data presented were statistically significant.

RESULT AND DISCUSSION

After setting the experiment, all two chambered MFCs were operated with different designated waste water Mediums at different conditions, as feed to support the formation of biomass and subsequent generation of electricity. The Microbial Fuel Cells were continuously monitored during experiment and readings were taken after each 24 hr, inoculation time was considered as time 0. Fuel Cells were operated for 15 days and readings were taken up to 10 days. Preliminary experiments conducted using MFCs showed that electricity could be generated using Sugar Industry waste water. Stable current output was achieved after three cycles.

When MFCs were inoculated with different designated Brewery waste water samples, there was about 24 h Lag phase followed by an increase in cell current. The initial increase of current here can be attributed to the presence of components that are easily utilized by *Saccharomyces cerevisae*. 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 5th day, after a drop in current was observed. A steady increase in current generation was observed with 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.

COD removal efficiency

During operation, all MFCs were continuously monitored for waste (as COD) removal to enumerate the potential of fuel cell to act as wastewater treatment unit. All MFCs showed their potential for COD removal indicating the function of *Saccharomyces cerevisae* in metabolizing the carbon source as electron donors (Table 1).

Under both temperatures, a decreased COD removal efficiency was observed when wastewater concentration was decreased from 50% to 25%. Medium A1 showed 68.42% and 58.38% COD removal efficiency at 35 and 45ºC, while Medium B1 exhibited 66.98% and 57.37% COD removal efficiency at 35 and 45ºC respectively (Fig. 1).

Similarly, Medium A2 showed 85.99% and 83.56% COD removal efficiency at 35 and 45ºC, while Medium B2 exhibited 81.73% and 80.44% COD removal efficiency at 35 and 45ºC respectively (Fig. 2).

Table 1. Current Generation and COD removal by different Mediums in Microbial Fuel Cells.

	Temp	Days	0	1	2	3	4	5	6	7	8	9	10
Mediuma 1	45°C	Current (mA)	0.00	0.12	0.39	0.96	1.35	1.86	3.46	4.32	5.29	5.01	4.28
		COD mg/L	1235	1178	1109	1014	962	892	833	746	672	560	514
		COD removal Efficiency (%)	0.00	4.61	10.20	17.89	22.10	27.77	32.55	39.59	45.58	54.65	58.38
	35°C	Current (mA)	0.00	0.64	0.98	2.12	2.38	2.96	4.80	5.29	6.38	6.72	5.49
		COD mg/L	1235	1205	1138	1078	936	893	798	737	609	517	390
		COD removal Efficiency (%)	0.00	2.42	7.85	12.95	24.21	27.69	35.38	40.32	50.68	58.13	68.42
Mediuma 2	45°C	Current (mA)	0.00	1.65	2.78	3.90	6.11	5.34	7.83	8.49	9.12	9.84	8.39
		COD mg/L	1235	1162	1086	867	828	730	624	512	444	299	203
		COD removal Efficiency (%)	0.00	5.91	12.06	29.79	32.95	40.89	49.47	58.54	64.04	75.78	83.56
	35°C	Current (mA)	0.00	1.98	3.13	4.28	7.45	6.37	8.96	9.48	9.97	10.45	8.89
		COD mg/L	1235	1132	1019	834	745	629	541	408	320	261	173
		COD removal Efficiency (%)	0.00	8.34	17.48	32.46	39.67	49.06	56.19	66.96	74.08	78.86	85.99
Mediumb1	45°C	Current (mA)	0.00	0.38	0.91	1.08	1.88	2.07	3.78	4..89	5.87	6.26	5.47
		COD mg/L	624	597	563	498	461	426	389	344	306	284	266
		COD removal Efficiency (%)	0.00	04.32	09.77	20.19	26.12	31.73	37.66	44.87	50.96	54.48	57.37
	35°C	Current (mA)	0.00	0.84	1.12	1.39	2.78	3.29	5.33	6.31	7.33	7.43	6.78
		COD mg/L	624	565	524	506	478	423	389	354	308	240	206
		COD removal Efficiency (%)	0.00	9.45	16.02	18.91	23.39	32.21	37.66	43.26	50.64	61.53	66.98
Mediumb 2	45°C	Current (mA)	0.00	1.28	1.74	2.91	4.26	3.52	5.95	6.97	7.89	8.27	7.79
		COD mg/L	624	591	549	479	419	378	332	276	239	167	122
		COD removal Efficiency (%)	0.00	5.28	12.01	23.23	32.85	39.42	46.79	55.76	61.69	73.23	80.44
	35°C	Current (mA)	0.00	1.42	2.38	3.19	5.18	4.49	6.88	7.69	8.54	8.13	7.98
		COD mg/L	624	586	524	476	413	326	299	218	187	161	114
		COD removal Efficiency (%)	0.00	06.08	16.02	23.71	33.81	47.75	52.08	65.06	70.03	74.19	81.73

Experimental data obtained, showing that Methylene Blue also influenced COD removal efficiency of the MFCs. MFCs containing Medium A2 and B2 (0.5% Methylene Blue solutions of 50 and 25% plain diluted waste waster) showed greater COD removal efficiency than that of MFCs containing Medium A1 and B1 (50 and 25% plain diluted waste water) (Figs. 3,4).

Time taken for carbon exhaustion was relatively less in Mediums containing Methylene Blue. This may be due to rapid electron transfer capacity of Methylene Blue which is used as mediator in Medium A2 and B2. Methylene Blue readily transferred electrons obtained by oxidation of the waste water, which ultimately helped in COD removal efficiency enhancement of the MFCs.

Figs. 1, 2, 3 and 4 clearly indicating that COD removal efficiency was also affected by temperature change. MFCs operated at 35ºC showed 2-10% greater COD removal efficiency than that operated at 45ºC. This may be due to *Saccharomyces cerevisae,* which is most active at temperature between 30-37ºC.

Current generation

Experimental data revealed the feasibility of current generation in conjugation with the wastewater treatment by all MFCs (Table 1). However, the performance and stabilization tendency with respect to current generation was found to be dependent on the substrate feeding rate.

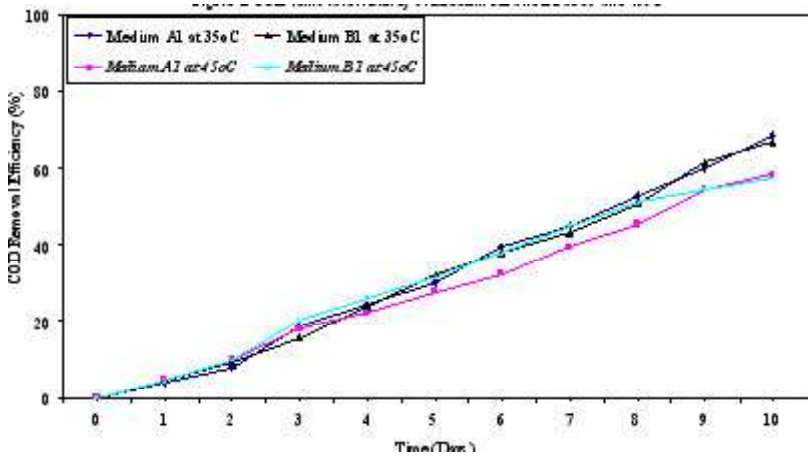

Fig. 1. COD removal efficiency of Medium A1 and B1 at 35 and 45°C

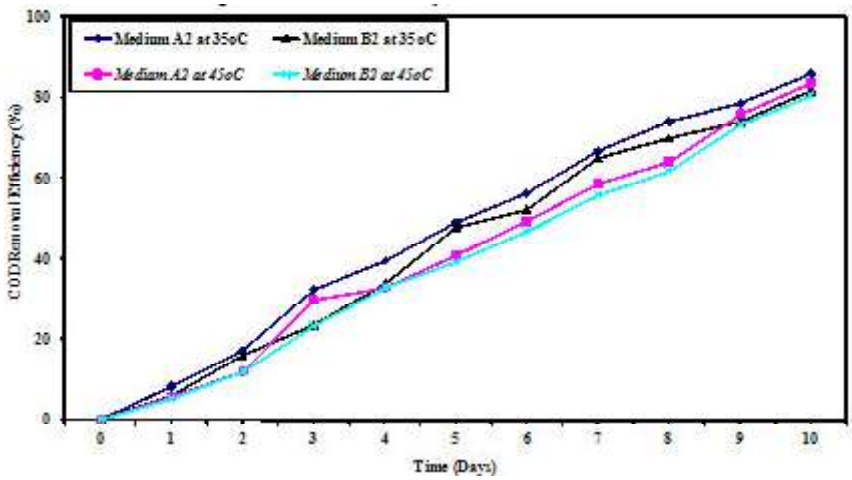

Fig. 2. COD removal efficiency of Medium A2 and B2 at 35 and 45°C

To evaluate the effect of temperature on MFCs performance, all MFCs were operated at 35 and 45°C. Experimental data indicate that performance of MFC was slightly decreased with increase of temperature from 35 to 45°C.

Fig. 5 shows the current generation by Medium A1 and A2 at 35 and 45°C. Both mediums started fermentation and current generation after about 24 hrs. Medium A2 showed maximum current generation at 35°C and reached a peak value of 7.45 mA after 4 days of operation. A decreased current observed after approximately 5 days which may be due to simpler substrate exhaustion in the Medium, but complex substrate were still present in the Medium, which generated low current. Current generation recovered quickly when 50% part of fresh Medium was replaced with a syringe through the anode and reached a maximum value of 10.45 mA after 9 days. Same current pattern was followed by Medium A2 at 45°C, which generated 6.11 and 9.84 mA current after 4 and 9 days of operation.

Medium A1 which was plain 2 times diluted waste water, also started current generation after about 24 hrs. But at both temperatures this culture did not showed any current fall. At both temperatures low yet continuous and maintained current generation was observed. This may be due to slow but continuous degradation of Sugar Industry waste

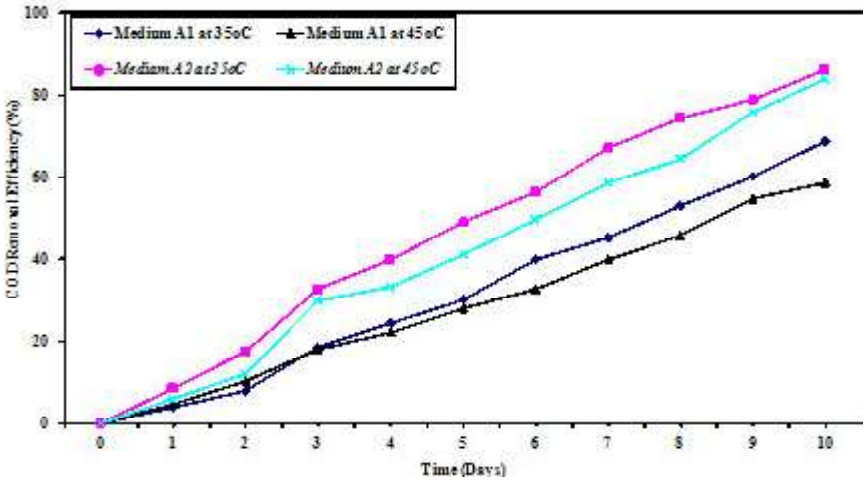

Fig. 3. COD removal efficiency of Medium A1 and A2

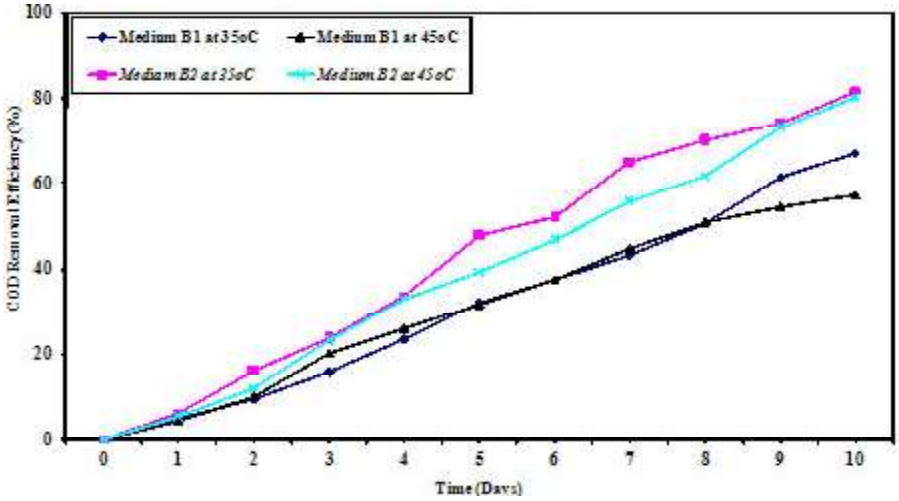

Fig. 4. COD removal efficiency of Medium B1 and B2

water by *Saccharomyces cerevisae*. As there was no rapid current generation (no rapid oxidation of substrate), readily oxidizable substrate were available to the *Saccharomyces cerevisae* upto a long time.

Fig. 6 shows the current generation by Medium B1 and B2 at 35 and 45ºC. Here also, both mediums started fermentation and current generation after about 24 hrs. Medium B2 showed maximum current generation at 35ºC and reached a peak value of 5.81 mA after 4 days of operation. Current decreased after 5th day of operation when readily oxidizable substrates were utilized in the MFC, but due to complex substrate a low current was still obtained. Current generation recovered quickly when 50% part of fresh Medium was replaced with a syringe through the anode and reached a maximum value of 8.54 mA after 8 days. Same current pattern was followed by Medium B2 at 45ºC, which generated 4.26 and 8.27 mA current after 4 and 9 days of operation.

Medium B1 which was plain 4 times diluted waste water, also started current generation after about 24 hrs. At both temperatures this Medium did not showed any current fall, yet at 35ºC it gave better results. At both temperatures low yet continuous and maintained current generation was observed. This may be due to slow but continuous degradation of Sugar Industry waste water by *Saccharomyces cerevisae*. As there was no rapid current generation (no rapid oxidation of substrate),

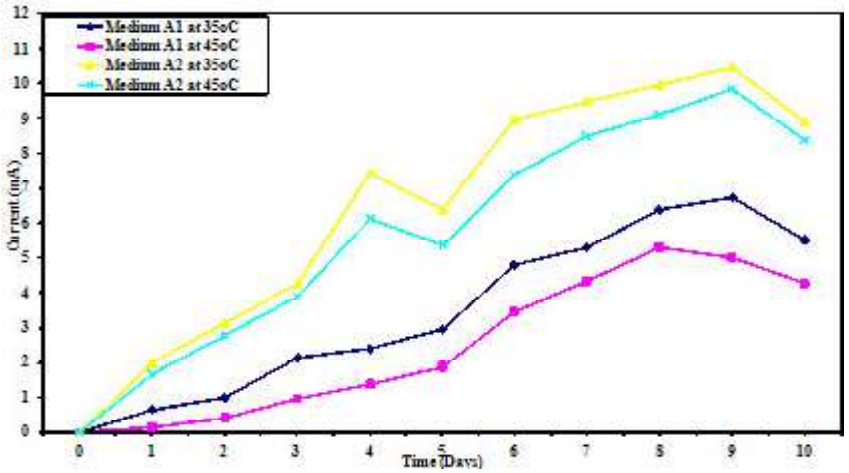

Fig. 5. Current Generation by Medium A1 and A2 at 35 and 45°C

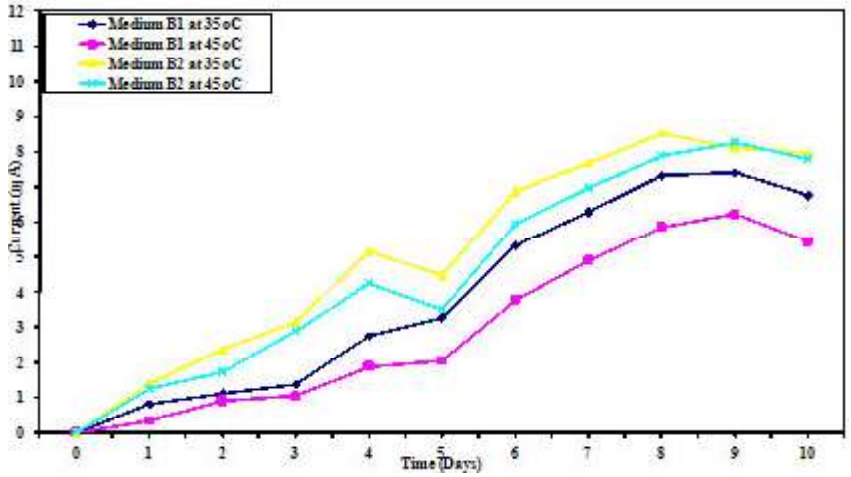

Fig. 6. Current Generation by Medium B1 and B2 at 35 and 45°C

readily oxidizable substrate were available to the *Saccharomyces cerevisae* upto a long time.

As shown in Figs. 5 and 6, that after addition of 0.5% Methylene Blue to the plain waste water Mediums, increased current production obtained throughout in Medium A2 and B2 which may be due to rapid electron transfer capacity of Methylene Blue. As the electrons were librated after oxidation of wastewater, those were captured by Methylene Blue and transferred to anode, which ultimately facilitated the increased current production.

To evaluate the effect of waste water concentration on electricity production, a comparative study was done with Medium A1, and A2 (plain and designed 2 times diluted waste water) also with Medium B1 and B2 (plain and designed 4 times diluted waste water) respectively.

Fig. 7 shows the current generation by Medium A1 and B1 at 35 and 45ºC. Surprisingly Medium B1 showed the superior results followed by Medium A1 at 35ºC. This may be due to easy availability of readily oxidizable substrates and less substrate inhibition in Medium B1. As Medium A1 and B1 were plain diluted waste waters, excess substrate in Medium A1 might cause substrate inhibition so ultimately less current generation by *Saccharomyces cerevisae.*

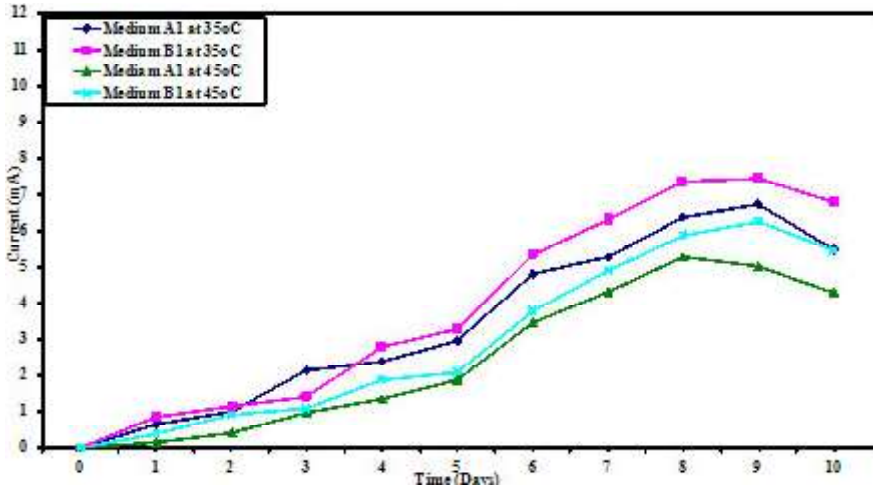

Fig. 7. Current Generation by Medium A1 and B1

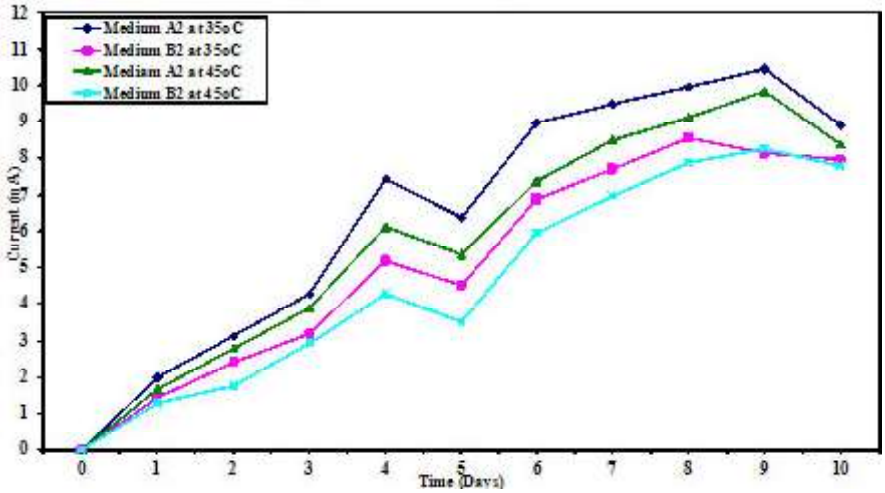

Fig. 8. Current Generation by Medium A2 and B2

Data clearly represents that under both temperatures; both Medium did not showed any major current fall. This may also due to poor yet maintained current generation by *Saccharomyces cerevisae*. Medium A1 approached a maximum current of 6.72 and 5.01 mA at 35 and 45ºC while Medium B1 achieved a maximum current of 7.43 and 6.26 mA at 35 and 45ºC after 9th day of operation.

Fig. 8 showed the current generation by Medium A2 and B2 at 35 and 45ºC. These Mediums generated typical results. Under both temperatures, an increased current generation was observed with increase of waste water concentration from 25% to 50%.

Medium A2 (0.5% Methylene Blue solution of plain 2 times diluted wastewater) showed maximum current generation at 35ºC and reached a peak value of 7.45 mA after 4 days of operation. Current decreased after 5th day of operation when readily oxidizable substrates were used up in the MFC. Meanwhile, degradation of complex components was taken place, so a low current was still obtained. Current generation recovered quickly when 50% part of fresh Medium was replaced with a syringe through the anode and reached a maximum value of 10.45 mA after 9 days.

Same current pattern was followed by Medium A2 at 45ºC, which generated 6.11 and 9.84 mA current after 4 and 9 days of operation. Medium B2 (0.5% Methylene Blue solution of plain 4 times diluted wastewater) achieved inferior current under both temperatures. Medium B2 generate maximum current of 4.49 and 8.54 mA after 4th and 8th day of operation at 35ºC, while at 45ºC 4.26 and 8.27 mA current was obtained after 4th and 9th day of operation.

CONCLUSION

Electricity was successfully generated from Sugar Industry waste water by *Saccharomyces cerevisae* using Microbial Fuel Cell technology. Temperature, Mediator and waste water concentration affected current generation and COD removal and by combining and designing the process by means of above parameters, increased electricity may obtain with treatment of wastewater.

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